COURTNEY M. PRICE
VICE PRESIDENT
CHEMSTAR

American Chemistry Council
Good Chemistry Makes It Possible

January 23, 2003

Dr. Michael Shelby
Director, CERHR
National Institute of Environmental Health Sciences
79 T.W. Alexander Drive
Building 4410, Room 103
P.O. Box 12233
MD EC-32
Research Triangle Park, NC 27709

Re: Comments of the Propylene Oxide/Propylene Glycol Panel
on Draft Expert Panel Report Propylene Glycol (NTP-CERHR-PG-02)

Dear Dr. Shelby:

The Propylene Oxide/Propylene Glycol Panel (PO/PG Panel) of the American Chemistry Council submits the attached comments and the review by Dr. Mark Udden, a clinical hematologist at Baylor College of Medicine, in response to the National Toxicology Program (NTP) Center for the Evaluation of Risks to Human Reproduction (CERHR) Draft Expert Panel Report (Draft Report) on propylene glycol. The member companies of the PO/PG Panel comprise the major domestic producers of propylene glycol in the United States.[1]

As explained briefly in this letter and detailed in the accompanying comments, the PO/PG Panel has serious concerns that the Draft Report does not adequately consider and weigh the substantial database available on propylene glycol which shows that over decades of use, propylene glycol has with few exceptions been remarkably free of reported adverse effects on humans. Indeed, the designation by the Food and Drug Administration (FDA) of propylene glycol as Generally Recognized as Safe (GRAS) for humans, 21 C.F.R. §184.1666, is fully supported by voluminous human and animal data. We believe considerable effort is needed to move the Draft Report from its current status to the quality NTP-CERHR should obtain.

To its credit, the FDA has studied the developmental effects of propylene glycol and, based in part on those results, has approved its use in literally hundreds, if not thousands, of prescription and over-the-counter pharmaceuticals used by millions in the U.S. alone. Propylene

[1] Members of the Propylene Oxide/Propylene Glycol Panel are The Dow Chemical Company, Huntsman Corporation, and Lyondell Chemical Company.

glycol has also been reviewed in 1994 by the Cosmetic Ingredient Review Expert Panel and approved for use in various cosmetic products. CERHR should be very wary of issuing a report that calls into question the adequacy of the available data or raises concerns about the safety of propylene glycol in vital medicines. Given its broad pharmaceutical uses, propylene glycol is unlike any other chemical reviewed by CERHR to date in that there are decades of extensive human exposure and widespread use in addition to numerous human studies and case reports.

Developmental Effects: The PO/PG Panel disagrees with the Draft Report's conclusions on the adequacy of the available developmental hazard information. The PO/PG Panel believes that existing data already demonstrate that propylene glycol is not a developmental hazard. Most notably, the Draft Report is inappropriately critical of the FDA developmental toxicity studies, conducted in four species. It is rare even for pharmaceutical agents to be tested to such an extent. Those studies provide reliable information that large doses of propylene glycol do not cause any developmental toxicity. Moreover, a recent, developmental toxicity study in the mouse provides even further additional evidence that propylene glycol is not a developmental hazard. Taken together, these data are sufficient to support a conclusion that propylene glycol does not present a developmental hazard to humans.

Inappropriately, the Draft Report contains speculation that the fetus would be adversely affected from lactate produced from propylene glycol metabolism, that any maternal acidosis would also cause acidosis in the fetus, and that due to the lack of metabolism of propylene glycol by the fetus/newborn/infant, more severe central nervous system (CNS) effects from propylene glycol may occur. Negative findings from animal developmental toxicity investigations support the conclusion that *in utero* exposure to these substances is of no toxicological consequence to the fetus.

Reproductive Effects: The PO/PG Panel believes NTP's own multi-generation reproductive study, supported by several earlier studies, provide adequate evidence to reach the conclusion that propylene glycol is not a reproductive hazard. Dr. Mark M. Udden also notes that the use of propylene glycol as a cryopreservative for human reproductive tissues, both sperm and oocytes, further shows the absence of reproductive concerns for propylene glycol.

Hematological Effects: Dr. Udden's report reviews in detail the literature on hematological effects of propylene glycol in both humans and the studied animal species. He concludes that hemolysis is not a significant factor in human risk assessment as it has only been observed with intravenous infusion in medical formulations with propylene glycol as a vehicle at very high concentrations (35-50%) and only when there is direct contact with the red blood cells, such as at the site of infusion. In animal tests, an enormous amount of propylene glycol in the diet is required to see any effects, and, in most cases only modest changes resulted. The cat is an exception as propylene glycol does produce significant Heinz body formation.

As a hospital-based hematologist, Dr. Udden reviewed the case reports and other human studies, noting that propylene glycol is usually well tolerated. Given the millions of patients worldwide who have been treated with propylene glycol-containing pharmaceuticals, the few reports of adverse effects show only what is obvious to the medical community – patients with impaired clearance capabilities and low birth weight infants must be closely monitored, as is also true with other aspects of their treatment. Simply stated, propylene glycol is an effective

excipient with little, if any, risk over a wide range of human exposures, with toxicity observations limited to a few special classes of patients who are typically under constant supervision in hospital Intensive Care Units.

General Toxicity: The report does not adequately characterize and evaluate the general toxicity and biological effects of propylene glycol for consumers and workers. The conclusion that propylene glycol poses only a low general toxicity hazard to the all members of the general populations is warranted based on available data. Although in medical usage propylene glycol may, in rare circumstances, contribute to adverse effects in some unhealthy individuals, these effects are difficult to interpret given the patients' pre-existing, and often multiple, diseases and other treatments that confound interpretation of the effects of propylene glycol itself and hence cannot be broadly applied to possible sensitive subgroups and certainly not to the general population.

Significantly, in several of the case-report studies reviewed by CERHR, the dosage applied was clearly contrary to label instructions. Abnormal organ physiology stemming from the disease processes, coupled with concurrent administration of toxic drug therapies, indicate results from studies in patients and burns trauma victims are an unreliable basis for predicting the toxicokinetics of propylene glycol in all population groups. In contrast to these few cases of adverse effects in clinical settings, the NTP-CERHR Draft Report must recognize and report the fact that millions of patients have been treated with propylene glycol-containing pharmaceuticals without experiencing adverse effects from the propylene glycol and with important health benefits. The Draft Report seems to overlook the fact that each year millions -- if not tens of millions -- of patients of all ages are administered propylene glycol-containing pharmaceuticals and monitored closely in hospitals around the world without detecting any adverse effects.

The data in the Draft Report do not show that propylene glycol causes lactic acidosis in unimpaired humans. Patients with impaired hepatic or renal function are at increased risk of toxicity from any substance that is cleared by the liver or kidney, not simply propylene glycol. As shown by its GRAS status and widespread use as a pharmaceutical excipient, the FDA, clinicians and pharmaceutical manufacturers have studied propylene glycol and reached the conclusion that it is an effective and safe component in many formulations. These pharmaceutical products are used only in clinical settings where effects on patients are closely monitored and appropriate risk-benefit decisions can be made. Since this is the first chemical that the CERHR has reviewed with extensive pharmaceutical use, it is perhaps understandable that the document fails to adequately recognize that the clinical studies and case reports provide limited utility when applied to general consumer and occupational exposures.

Thus, the data presented in the Draft Report do not support a view that there exists a sub-population sensitive to propylene glycol. Pre-existing disease states, coupled with concurrent administration of toxic drug therapies, clearly explain the findings for many, if not all, of these studies. Indeed, CERHR may want to seek more input from the FDA and the medical community to put these data into proper context. Dr. Udden's report is an important step in that direction.

Exposure Assessment: The existing occupational and consumer product information is sufficient to conduct a screening level exposure assessment and an evaluation of risk for the

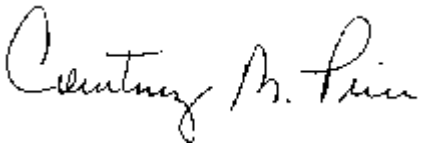
demonstrated very low hazard substance propylene glycol. The PO/PG Panel is surprised that CERHR did not include an adequate screening level exposure assessment as part of its Draft Report. Experienced exposure assessors could develop an exposure assessment from available estimates of human consumption and databases maintained by EPA and others. Given the very low hazards demonstrated for propylene glycol, a screening level exposure assessment is all that is needed to draw risk conclusions. As noted previously, propylene glycol is widely used and has a long history of use with only very few reports of adverse effects, empirically demonstrating that propylene glycol poses very low risk to humans.

* * * * *

In sum, the data review presented in the Draft Report has many errors and omissions that likely result from the abbreviated report development time available for propylene glycol. For example, as explained in our detailed comments, the evaluation of human and animal skin irritation data in the Draft Report is inaccurate and requires substantial revisions. As pointed out in the PO/PG Panel's comments of May 5, 2002, the NTP's CERHR decided to review propylene glycol without following its own priority-setting procedure, and in a much shorter time frame than allotted to previous and concurrent reviews. This shortened approach may have deprived CERHR from full consideration of whether a CERHR evaluation of the reproductive and developmental toxicity of propylene glycol is an appropriate use of limited NTP resources and the resources of others involved. Nonetheless, at this point, clearly more of these resources must be applied to improve the Draft Report to the level needed for a quality review of propylene glycol. We further suggest that CERHR seek assistance from experienced clinicians given the importance of propylene glycol in pharmaceuticals and the data obtained from medical usages.

If you or your staff has any questions, please contact the PO/PG Panel Manager, Dr. Anne P. LeHuray at (703) 741-5630 or *anne_lehuray@americanchemistry.com*.

Sincerely yours,



Courtney M. Price
Vice President, CHEMSTAR

Enclosures

U.S. NATIONAL TOXICOLOGY PROGRAM (NTP)
CENTER FOR THE EVALUATION OF RISKS TO HUMAN REPRODUCTION (CERHR)

NTP-CERHR EXPERT PANEL REPORT ON THE REPRODUCTIVE AND DEVELOPMENTAL TOXICITY OF PROPYLENE GLYCOL

DECEMBER 2002

NTP-CENTER-PG-02

COMMENTS OF THE PROPYLENE OXIDE/PROPYLENE GLYCOL PANEL OF THE AMERICAN CHEMISTRY COUNCIL

COMMENTS SUBMITTED: JANUARY 23, 2003

SCHEDULED EXPERT PANEL REVIEW DATE: FEBRUARY 11 - 13, 2003

INTRODUCTION

The Propylene Oxide/Propylene Glycol Panel (PO/PG Panel) of the American Chemistry Council (ACC) submits these comments in response to the National Toxicology Program (NTP) Center for the Evaluation of Risks to Human Reproduction (CERHR) notice (Federal Register 67:236, Dec. 9, 2002) indicating that the CERHR has convened an "Expert Panel" and released a Draft Expert Panel Report on Propylene Glycol (Draft Report). The Expert Panel is scheduled to meet February 11 through 13, 2003 to further discuss the Draft Report.

For reasons explained in detail in these comments, the PO/PG Panel agrees with the conclusion that propylene glycol is not a reproductive hazard and disagrees with the view expressed in the Draft Report that the available information is inadequate to reach a conclusion about developmental toxicity. This finding of "inadequacy" is based on unwarranted criticism of developmental toxicity studies conducted by the Food and Drug Administration (FDA) in four different species, the results of which are supported by results of a modern developmental toxicity study. The criticism of the FDA studies reflects a bias towards finding adverse effects that is woven through the Draft Report regardless of whether such effects are supported by a careful analysis of the data.

The Draft Report does not acknowledge the long history of worldwide use of propylene glycol in food, prescription and non-prescription drug products, cosmetics, toiletries, soaps, and personal care products of many varieties. More than sufficient quantitative and empirical information is available to perform a screening level exposure assessment that can be used to draw risk conclusions. Such an assessment would show that, although propylene glycol has been widely used in consumer end-use applications for many years, there have been only a very few reports of adverse effects, some of which, as discussed in these comments, are likely unrelated to propylene glycol exposures. The history and pattern of propylene glycol use supports the empirical conclusion that exposure to propylene glycol poses a very low risk to humans. Indeed, propylene glycol has a long history of beneficial uses as a pharmaceutical excipient, helping to deliver life-saving and life-enhancing drugs to very sick people.

These comments contain detailed discussion of the reviews and conclusions presented in the Draft Report. To help understand some of the information presented in the Draft Report, the PO/PG Panel obtained a review by Dr. Mark Udden, a clinical hematologist at Baylor College of Medicine. Dr. Udden's remarks are incorporated in the following comments and his full report is included as an attachment. In a separate submission, after release of the Draft Report, the PO/PG Panel provided an unpublished developmental toxicity study (Driscoll *et al.*, 1993) to the CERHR. A robust summary of this report, in the internationally agreed IUCLID database format, is also included as an attachment. The PO/PG Panel's comments refer to an unpublished study (Quast *et al*., 1979) that was reviewed by international governments as part of the Organization for Economic Cooperation and Development (OECD) review process (reference 4). The robust summary for the Quast *et al.* (1979) study is included as Attachment C.

COMMENTS

1. Sufficient information is available to evaluate and conclude that propylene glycol is not a developmental hazard.

1.1 The Draft Report is inappropriately critical of the FDA developmental toxicity studies.

The Draft Report is inappropriately critical of the FDA studies conducted with propylene glycol for developmental toxicity, which provide developmental toxicity data on four different species. For instance, the Draft Report cites a lack of detailed information on malformations. However, the incidence of malformation in each species studied is very small. In many instances, a single fetus is affected. It is reasonable to question what greater detail would be necessary. It is rare even for pharmaceuticals to be tested to this extent. To have data in four species, none of which indicates any potential for developmental toxicity, is greater proof of a lack of effect than exists for most other chemicals, including some that have already been considered by the CERHR process (or its predecessor IEHR process) to have an adequate database.

While the lack of maternal body weights and feed consumption data over the treatment period does not meet current guidelines, the important final maternal body weights are presented, showing no difference between the propylene glycol exposed groups and the control group. Any dramatic differences in body weight, body weight gain, or feed consumption would have been reported and would have been manifest in final body weights.

Although detailed experimental methods are not presented, the procedure for conducting these studies at the time (late 1960s and early 1970s) was straightforward and can be assumed from the protocols of the day. The propylene glycol manufacturing process has not essentially changed since that time, although continuous incremental improvements have been made. While the overall quality is likely better today, the impurity profile is similar to propylene glycol made in the past. Thus, the test material used in the FDA studies would have been of similar purity, and stability to that manufactured today, and it would be appropriate to assume the propylene glycol tested in the FDA studies is similar in properties to the propylene glycol available today.

In terms of the sequence of the necropsy, it appears from the stated methods that with the exception of the rabbits, all of the pregnant females were mated in house and assigned to a dose group prior to initiation of treatment. Since laboratory animals typically do not all breed on the same night, they would not all be necropsied on the same day. Therefore, with much smaller numbers being necropsied each day, the sequence of necropsy becomes much less important (although it is agreed with the main point of the presentation in the Draft Report that the necropsy is best conducted either in a random or stratified fashion to prevent all of one dose group being done and another following 6-8 hours later).

On page 56 of the Draft Report, a large number of wavy ribs in the rat study is discussed but in the Table 3-4, there is no mention of wavy ribs. In addition, the claim is made that the 1600 mg/kg bw propylene glycol rats have incomplete ossification of the vertebrae at the same frequency as the positive control. According to Table 3-4, the data (fetuses/litters) for that parameter in the sham control was 0/0, in the positive control was 101/19 (137/20 examined), and in the 1600 mg/kg bw group was 18/9 (180/24 examined). According to these data, the

incidence (fetus/litter) in the positive control was 74%/95% and the 1600 mg/kg bw group was 10%/38%. The incidence is hardly "similar" between these two groups and the Draft Report should be corrected to properly reflect the data.

The statement is also made that in the mouse study, the incidence of malformations across all groups is "large" and raises concerns about the validity of the study. This statement is not supported by the data. There are only three soft tissue abnormalities reported in all of the mice fetuses and the majority of the findings in the skeletal data are "incomplete ossification" (not surprising since the dams were killed on gestation day 17). Many of the other skeletal findings would be properly classified as variations and not malformations. The incidence of these variations in the skeletal findings in the mice are similar to what is currently observed in mouse teratology studies and represent the normal variation in development in the species.

The same misstatement regarding the rat rib and vertebrae data is repeated on page 57 of the Draft Report, and needs to be corrected.

The Union Carbide mouse study (Driscoll *et al.*, 1993) described below and in the attached robust summary, should alleviate the concerns expressed in the Draft Report. However, the review presented in the Draft Report assumes that some "important observations" may have been missed in the FDA studies and therefore expresses "uncertainty" about the data and feels it is insufficient to predict human health effects. It is important to remember the time period in which these studies were conducted. The thalidomide disaster had occurred about ten years earlier and a definitive link between drug exposure and adverse developmental outcome had been proven. Many of the scientists in the field of teratology at that time had received "hands-on" training from Jim Wilson and other pioneers in the field. The regulatory and scientific community was on "high alert" for drugs and chemicals that caused birth defects. It is against this background that this teratology study, investigating in four separate species, was conducted at the request of a major US Agency. It is difficult to imagine that a finding or pattern of defects occurred that was overlooked in this study with propylene glycol, a material that was to be used in intravenous solutions and drugs for humans. Perhaps more confidence should be afforded the pioneers of the field and their data, and less skepticism given to studies simply because they are old. After all, the basic design and conduct of these studies have not appreciably changed, and the ability of toxicologists and pathologists experienced in this field to detect malformations is the same in 2002 as it was in 1970.

In terms of the lack of statistical analysis, it is obvious that the majority of the results are not statistically significantly different from the control values. There are a few instances where a statistical analysis would be helpful, but there is no overall pattern of an effect, either within a species or within an organ system. Also, the lack of effect on fetal body weights suggests that no developmental toxicity occurred at the doses tested, which significantly exceeded what current guidelines would dictate. Usually, fetal body weights are the most sensitive indicator of an adverse effect in these studies. Finally, there are decades of experience with the species and strains of animals that were used in the FDA studies, allowing anyone with experience in developmental toxicity testing to conclude that none of the results presented for propylene glycol are indicative of a biologically meaningful effect.

On page 58 of the Draft Report, the review laments the lack of a dose response in the FDA studies. Perhaps the lack of the dose response should instead be applauded since there were no adverse effects found with quite high propylene glycol exposures.

1.2 A second developmental toxicity study conducted in the mouse is available that provides even further evidence that propylene glycol is not a developmental hazard.

A mouse teratology study with propylene glycol was conducted by Union Carbide Corporation (now The Dow Chemical Company) at Bushy Run Research Center in 1993 (Driscoll *et al.*, 1993). The study was well-conducted and essentially performed to regulatory guidelines and in compliance with GLPs. A copy of the study report has been provided to NTP CERHR. The following is a summary of the study methods and results.

Propylene glycol (99.9% purity) was administered by gavage to CD-1® mice (30/group) from gestation day (GD) 6 through 15 (GD 0 = day of sperm positive finding). The test article was administered undiluted and the dose levels were 0.5, 5.0, or 10 ml/kg bw/day (518, 5,180, or 10,360 mg/kg bw/day) with the vehicle control receiving water at the same dose volume as the high-dose group. Clinical observations were collected daily (twice daily during the dosing period) and maternal body weights, feed, and water consumption was measured over three-day intervals. The dams were euthanized on GD 18. Maternal parameters collected at necropsy included final body weights, liver and kidney weights, gravid uterine weights, corpora lutea, and number and status of implantation sites. All live and dead fetuses were weighed, sexed, and examined externally for malformations (including cleft palate) and variations. All of the live fetuses were examined for thoracic and abdominal malformations and variations by the method of Staples (1974). One-half of the liver fetuses were decapitated, heads fixed in Bouin's solution, and craniofacial structures examined by the method of Wilson (1965). All fetuses were eviscerated, fixed in ethanol, and processed for skeletal examination. Alizarin red S was used to stain the skeleton for examination (Peltzer and Schardein, 1966). The statistical unit for comparison was the litter, and data were analyzed using Levene's test for equality of variances, analysis of variances (ANOVA), and t-tests, as appropriate.

There were no treatment-related effects on maternal parameters other than an increase in water consumption in the 5.0 and 10.0 ml/kg bw/day groups. Maternal parameters collected at necropsy revealed no treatment-related effects. At necropsy there were 28 to 29 live litters per group available for examination. There were no treatment-related differences in the total number of implantations, number of viable or nonviable implants, or sex ratios. The incidence of external, visceral, and skeletal malformations/variations was comparable between the treated and control groups. There were no statistically significant differences in male or female fetal body weights between the treated and control groups. However, when the fetal body weight data for the sexes were combined, there was a 3% decrement (statistically significant) in fetal body weights in the 10 ml/kg bw/day group when compared to the control group values. The authors considered this decrement in body weight not to be biologically significant due to the small magnitude of the change and the fact that the low dose group had similar fetal body weights (lack of a dose- response with a 20-fold increase in dosage between the low and high dose groups). An increase in a variation (unossified cervical centra #1, #2, #3, #4) in the 10 ml/kg bw/day group was not considered biologically significant since the incidence was within the historical control litter incidence range (7 – 23%). Two unrelated findings in the low-dose group

were not considered treatment-related due to the lack of dose-response relationship. The authors note that they expected 4 statistically significant changes in the 86 individual endpoints evaluated. The authors concluded that 10 ml/kg bw/day (10,360 mg/kg bw/day) was the No Observed Effect Level (NOEL) for developmental toxicity of propylene glycol in mice.

The FDA study used dose levels of 16, 74.3, 345, and 1,600 mg/kg bw/day propylene glycol, and the Union Carbide study used dose levels of 518; 5,180; and 10,360 mg/kg bw/day. The high dose group in the FDA study had fetal body weights (sexes combined) that were 6% greater than the control group; this difference was not statistically significant. While it is not easy to compare these two studies due to the large differences in the dose levels used, it does illustrate the range of differences (both increases and decreases) in mean fetal body weights typically observed in these studies.

Clearly, the quality and results of the Union Carbide study provide additional support to the FDA study reports that the CERHR has concluded are insufficient for estimating risk to humans for developmental toxicity of propylene glycol. Together, the weight-of-evidence indicates that propylene glycol does not pose a risk for developmental toxicity in humans and the CERHR report should adopt this conclusion.

1.3 Speculation that the fetus would be adversely affected by lactate produced from propylene glycol metabolism, that any maternal acidosis would also cause acidosis in the fetus, and that due to the lack of metabolism of propylene glycol by the fetus/newborn/infant, more severe CNS effects from propylene glycol may become manifest is unwarranted. Negative findings from animal developmental toxicity investigations support the conclusion that in utero exposure to these substances is of no toxicological consequence to the fetus.

On the last paragraph of page 20 of the Draft Report, under the section titled "Utility (adequacy) for CERHR evaluation process," the reviewers speculate regarding the proximate toxicant for propylene glycol. Included in the speculation is that the fetus would be adversely affected by lactate produced from propylene glycol metabolism, that any maternal acidosis would also cause acidosis in the fetus, and that due to the lack of metabolism of propylene glycol by the fetus/newborn/infant, more severe CNS effects from propylene glycol may become manifest.

It is important to note that propylene glycol has not been demonstrated to cause any developmental toxicity in any laboratory species or humans to date, even at doses exceeding 10,000 mg/kg bw/day. Therefore, the need to identify a "proximate toxicant" is of questionable value since there is no toxicity for the "proximate toxicant" to identify with. Any concerns regarding D- or L-lactate affecting development should be alleviated with this information.

It is also important to realize that during embryogenesis, the conceptus itself produces large amounts of lactate that diffuses across the placenta into the maternal circulation where the maternal liver converts it back to pyruvate (Braunwald *et al.*, 2001). Therefore, under normal circumstances, the embryo is exposed to plenty of endogenous lactate during development. The additional lactate present from propylene glycol metabolism would be dwarfed by the amount present from endogenous metabolism. Another physiological event that naturally produces large amounts of lactate is exercise. Lactate produced from muscle work diffuses into the blood and is

converted by the liver to pyruvate. If we were concerned about lactate affecting development, then there should be concern regarding exercise during pregnancy. On the contrary, exercise during pregnancy is encouraged. The ability of the maternal system to maintain normal blood pH and lactate levels in the face of this increased lactate production suggests very good functional reserve capacity to cope with this phenomenon. The amount of lactate added to this system from propylene glycol metabolism would appear to be extremely small compared to normal endogenous production, especially during pregnancy.

2. The report does not adequately characterize and evaluate the general toxicity and biological effects of propylene glycol. The available information supports the conclusion that propylene glycol poses low general toxicity hazard to all members of the general population. Although with extreme exposures, propylene glycol may contribute to the development of adverse effects in some unhealthy individuals, these effects are difficult to interpret given their pre-existing diseases and other treatments and hence can not be universally applied to 'possible sensitive subgroups' and certainly not to the general population.

2.1 Abnormal organ physiology, coupled with concurrent administration of toxic drug therapies, indicates that results from studies in patients and burns trauma victims are an unreliable basis for predicting the toxicokinetics of propylene glycol in the general population.

Much of the information on the pharmacokinetics of propylene glycol in humans, presented in the Draft Report, has been obtained from studies with patients or burns trauma victims receiving medication which contains propylene glycol as an excipient. This includes both case reports (generally short, *ad hoc* observational studies with no pre-planned scientific objective) and experimental studies (which include measured data obtained by following an investigative protocol with appropriate controls and statistical analysis of the data). In general terms, the former can be considered *hypothesis generating* while the latter are *hypothesis testing* indicating that that there will be differences in the scientific reliability and generality of the conclusions obtained

It is also important, with both types of study, to assess objectively whether factors such as disease state, physical trauma or pharmacologically active ingredients present in the medication may have influenced the outcome. In other words, some degree of "expert judgment" is needed to determine if the metabolism and disposition of propylene glycol in *patients* (often with severely compromised and/or debilitated health status) is comparable to that expected for *normal* subjects.

To illustrate these points, some of the human studies currently included in the Draft Report are briefly reviewed below.

Arbour and Esparis (reference 42) reported the occurrence of "osmolar gap metabolic acidosis" in a 60-year old man treated for hypoxemic respiratory failure. This individual required positive-pressure ventilation to maintain vital signs, and was therefore administered an 'escalating infusion' of Lorazepam in a vehicle containing 40% propylene glycol (the lorazepam was necessary for the patient to tolerate endotracheal intubation). As a consequence of this regimen, the patient received, by intravenous infusion, a total of 540 g propylene glycol along with the Lorazepam over 5 days, resulting in "*deep sedation*". Serum propylene glycol was 780 mg/l, while other laboratory measurements led to a diagnosis of osmolar gap metabolic acidosis at the end of the 5-day period. Faced with this diagnosis, Lorezapam treatment (and, hence, infusion of propylene glycol) was immediately discontinued and serum parameters returned to normal within 72 hr. Given the "deep sedation" needed to intubate/ventilate this patient, it seems possible that renal and other organ functions involved in clearance of propylene glycol may have been compromised. In any event the abnormal physiological status of this subject (*i.e*., presence of pre-existing hypoxemic respiratory failure), coupled with the massive intravenous dose of propylene glycol that was administered, suggest that this short case report is of limited reliability in an assessment of the toxicokinetics of propylene glycol.

Glasgow *et al.* (reference 30) reported an increased osmol gap in 11 infants receiving a vitamin preparation (MVI-12) by intravenous injection. From graphical data presented in this report, serum propylene glycol was in a range of approx. 600-10,000 mg/l. The body weight of the infants was 1,000-4,500 g with 6 infants weighing less than 2,000 g (*i.e*., indicative of premature birth). All infants were given a single 10 ml injection daily for at least 5 consecutive days, with no adjustment for body weight. It is unsurprising that the highest serum levels of propylene glycol were found in the premature, lower weight infants, whose intravenous dose of propylene glycol exceeded 5,000 mg/kg bw up to a maximum of 10,400 mg/kg bw. When discussing their findings, the authors note that "*Communication with other centers indicates that the dose of the multivitamin preparation may be higher than that generally used*" and that "*the package insert indicates that MVI-12 is intended for patients 11 years of age and older.*" This suggests that toxic hypervitaminosis, with probable impairment of hepatic and/or renal functions, would have contributed to the outcome of this investigation. The uncertain physiological and metabolic status of these infants indicates that results from this multiple-case report are unreliable and are of little, if any, relevance to a prediction of the toxicokinetics of propylene glycol in normal infants.

Kulick *et al.,* (reference 28) examined the side effects associated with the use of silver sulfadiazine treatment in a *prospective* experimental investigation involving 45 patients with second or third degree burns affecting at least 20% of their total body surface area. The patients were studied over 30 months, during which time 14 individuals died. The precise treatment regimen used is not clearly stated, however the minimum dose of silver sulfadiazine cream applied was 800 g/patient/d, while the (theoretical) maximum was 10,800 g/patient/d. The propylene glycol content of the cream was not stated, however propylene glycol was detected in serum from 53% of the patients (max. 1,300 mg/l serum in living patients, max. 9,800 mg/l in deceased patients). There was a significant association between serum propylene glycol concentration and increased serum osmolality and osmolal gap ($P<0.0005$ in both instances). However the authors present other information which demonstrates that kidney function was adversely affected in the patients. Biopsies from 11 of the 14 patients that died showed clear

histopathological evidence of kidney damage (post-mortem degeneration precluded examination of tissue from the remaining decedents), with positive fluorescence staining for immune complexes to sulfadiazine on the glomerular membrane in 5 samples. Serum from 18 of 20 subjects revealed strong immune reactivity toward sulfadiazine (determined using counterimmunoelectrophoresis) and were also positive for immunoglobulins active toward sulfadiazine (predominately IgG). (Interestingly, similar methods were also applied to propylene glycol in this investigation, but no immune complex or antibodies were detected.) When discussing these findings, the authors note that sulfadiazine is an *established renal toxicant* and has a recognized potential to induce allergic reactions. They conclude that their data are consistent with enhanced absorption of propylene glycol across burned skin, leading to high serum (and urine) levels. They also note that the hyperosmolal state present in the majority of these patients was not explained solely by propylene glycol and that "other factors" (including tissue breakdown components present as a consequence of burns trauma) were involved. Although not explicitly mentioned. this presumably also includes incipient renal failure caused directly by sulfadiazine. Hypovolemic shock due to loss of dermal intergrity is another likely contributor, given the high percentage of skin surface involved. Since it is not possible to disentangle the relative contribution of very high serum levels of propylene glycol, shock related to burns trauma and sulfadiazine-induced renal impairment in the etiology of the hyperosmolality noted in this study, the reliability and utility of these data in any evaluation of the toxicokinetics of propylene glycol appears limited.

Fligner *et al.* (reference 29) reported hyperosmolality in an 8-month old infant following treatment with silver sulfadiazine. The patient was initially treated for deep second and third degree burns on the chest (8% of total body area) which subsequently (and unexplainedly) progressed after 10 days to toxic epidermal necrolysis affecting 70% of the total body surface area. The condition was said to be equivalent to *second-degree burns*. Propylene glycol was present as an excipient in the silver sulfadiazine cream used a medication, and a maximum serum concentration of >10,000 mg propylene glycol/l serum was reported. There was a positive relationship between serum propylene glycol and osmolal gap. Since the maximum serum concentration of propylene glycol in this study (10,000 mg/l) was similar to that found in the decedent subjects from the study by Kulick *et al.* (9,800 mg/l), it is likely that the internal dose of sulfdiazine was also comparable between the two investigations. Similar degrees of sulfadiazine-related renal impairment would therefore be anticipated in the two studies. Again, metabolic and physiological anomalies unrelated to propylene glycol administration suggest that the information presented in this case study is unreliable and does not provide a sound basis for an evaluation of the toxicokinetics of propylene glycol *in vivo*.

An additional point to consider when assessing information from burns trauma patients is the potential confounding influence of hypovolemic shock on renal function in burns trauma victims. This term refers to the commonly observed reduction in plasma volume seen in individuals with third degree or extensive second degree burns (Behrman *et al.*, 2000; Townsend *et al.*, 2001). It is associated with acidosis (arising from poor tissue perfusion and independent of any external chemical agent) and increased plasma osmolality (triggered by the release of intracellular proteins, *etc*., into the circulation from damaged tissue, plus reduced clearance as a consequence of impaired renal function). These changes further hinder the clear interpretation of results obtained by Kulick *et al*. and Flinger *et al*.

Yu *et al.* (reference 23) investigated the pharmacokinetics of propylene glycol in humans following a multiple oral dosing schedule. The subjects received either 20.7 g of propylene glycol every 8 hr (equivalent to 62.1 g/d), or 41.4 g every 12 hr (equivalent to 82.8 g/d), both for a minimum of 3 d. In addition to propylene glycol, the preparation contained (among other ingredients) 7.25 ml (approx. 5.8 g) of alcohol-USP leading to an estimated received ethanol dose of 17.4 g/d or 23.2 g/d, respectively. As discussed in detail elsewhere in the Draft Report, both propylene glycol and ethanol are substrates for ADH. It is therefore probable that competitive inhibition would have affected the pharmacokinetic results obtained from this study, influencing their reliability and accuracy.

Conclusions pertaining to the toxicokinetics of propylene glycol based on results of references 20, 28, 29, 30, 42 should therefore be removed from revisions of the Draft Report. Other, more reliable data are cited which can be used to assess the disposition of propylene glycol *in vivo*. This includes human volunteer studies reported by Kolloffel *et al.* (reference 24; intra-rectal) and Speth *et al.* (reference 25; intravenous) together with numerous investigations in animals.

2.2 Evidence that propylene glycol causes lactic acidosis in normal humans is lacking.

A significant portion of Section 2.1.3 (Metabolism) of the Draft Report is devoted to the possible occurrence of *lactic acidosis* in humans and animals following exposure to propylene glycol. Two key experimental studies (Christopher *et al.* (1990; reference 33) and Morshed *et al.* (1991; reference 34)) are used in the Draft Report to support this hypothesis.

Christopher *et al.* (1990) measured the concentration of D- and L-lactate in serum from cats fed a control diet or test diets containing 12% or 41% propylene glycol (designed to deliver 1.6 or 8.0 g propylene glycol/kg bw/d, respectively), both considerably higher than current guideline recommendations of maximum 5% test substance in diet (40 CFR 798.3260; 40 CFR 798.3300). They recorded a dose-dependent increase in D-lactate over the 35 days of the study: maximum of 1.96 mmol/l in the 'low' dose group (calculated to be equivalent to 180 mg/l lactic acid) and maximum 7.12 mmol/l in the 'high' dose group (equivalent to 640 mg/l lactic acid). Levels of L-lactate decreased. When discussing their findings, the authors comment that "D-lactic acidosis of the magnitude observed in the cats of this study has been observed in humans after gastrointestinal bypass operations…" but provide no evidence for any causal association with propylene glycol. For reference, *Harrison's Principles of Internal Medicine* (Braunwald *et al*., 2001) notes that the normal range for lactate in adult human venous blood is 50-150 mg/l. Thus blood lactate values in cats following repeated ingestion of a diet containing 8% propylene glycol (1.6 g propylene glycol/kg bw/d) are just above the normal human range, while values in cats ingesting a diet of 41% propylene glycol (8 g/kg bw/d) are much above the human range.

Morshed *et al.* (1991) followed the concentration of L- and D-lactate in venous blood of rabbits for 3 hr following gavage administration of 38.66 mmol propylene glycol/kg bw (equivalent to 2.94 g/kg bw). The maximum concentration of propylene glycol in blood (41.04 mmol/l, equivalent to 3,120 mg/l) was achieved 1 hr post-dosing. The concentration of L-lactate peaked at 0.25 hr (2.55 mmol/l, equivalent to 230 mg lactic acid/l; increased 2.4-fold (significant) relative to fasted control), then was decreased to 1.7-fold above control by 3-hr post-dosing. D-lactate blood level was maximal at 3 hr (0.15 mmol/l, equivalent to 13 mg lactic acid/l; increased 30-fold (significant) relative to fasted control). When discussing these findings

the authors note they are indicative of hyperlactatemia (that is, a simple increase in blood lactate) and *not* lactic acidosis (increased blood lactate resulting in a decrease in blood pH and with adverse physiological and/or metabolic consequences). This is an important clinical and toxicological difference, which is not treated in a consistent manner in the Draft Report. The concentration for L-lactate (230 mg/l) was increased compared with the human 'normal' range following bolus administration of 2,940 mg propylene glycol/kg bw.

Despite extensive discussion of lactic acid acidosis, the Draft Report contains only one citation in support of the contention that this is an issue for humans: this is the case study from Arbour and Esparis (reference 42) who reported "osmolar gap metabolic acidosis" in a 60-year old man treated for hypoxemic respiratory failure. Serum lactic acid levels were measured, and found to be 258 mg/l. While this is higher than the upper normal bound of 150 mg/l given in *Harrison's Principles of Internal Medicine* (Braunwald *et al.*, 2001), it is consistent with hyperlactatemia and *not* lactic acid acidosis (following criteria of Morshed *et al.*). Other information presented in Oh *et al.* (1985; reference 48) confirms that conclusion. These authors investigated metabolic utilization and renal handling of lactic acid in 10 male volunteers infused with a racemic mixture of D- and L-lactate for 150 minutes. The maximum plasma concentration of D-lactate was 4.5 to 6.0 meq/l (equivalent to 500-670 mg/l), while L-lactate peaked at 4.0-6.7 meq/L (equivalent to 450-750 mg/l). By inference, the peak plasma concentration of *total* lactate would have been 950-1420 mg/l. The authors note that infusion of D,L-sodium lactate must have caused volume expansion and increased urine volume (resulting from sodium bicarbonate and sodium lactate diuresis), however no adverse clinical effects were noted.

It is also noted that while D-lactate appears to predominate in the cat following sub-acute administration of propylene glycol, and L-lactate predominates in the rabbit after acute exposure, it is not possible (on the basis of two studies of differing duration) to determine whether this reflects a species difference in propylene glycol disposition/metabolism.

In summary, while animal data demonstrate that blood lactate is increased following ingestion of multi-gram amounts of propylene glycol, published data indicate that adult humans tolerate high blood lactate levels with no apparent short-term consequences. This suggests that lactic acidosis is unlikely to be an issue for the general population or in the majority of patients exposed to propylene glycol.

2.3 The role of renal clearance in removing propylene glycol from the body is inaccurately and inconsistently discussed in the Draft Report.

The role of renal clearance in removal of propylene glycol from the body is first discussed in the metabolism section of the draft report (page 12, paragraph 3). Renal clearance is 45% in humans (Arbour and Esparis, 2000; reference 42), 55-88% in dogs (Ruddick, 1972; reference 43, citing Lehman and Newman, 1937; reference 36) and 2.4-14.2% in rabbits (Yu and Sawchuck 1987; reference 44) and 2-17% in rats (Morshed *et al.*, 1988; reference 35). This information demonstrates that in some species (in particular humans and dogs) the kidney makes a significant contribution to removal of propylene glycol from the body. It is unknown, therefore, why the Draft Report subsequently states (page 24, paragraph 2) that "Very little propylene glycol is cleared by the kidney …": this is clearly not the case.

2.4 Patients with impaired hepatic or renal function are at increased risk of toxicity from any substance that is cleared by the liver or kidney, not simply propylene glycol.

Results obtained by Yu *et al.*, 1985 (reference 23) demonstrate that hepatic clearance (metabolism) of propylene glycol is saturated at high plasma concentrations (1,200-3,700 mg/l) leading to an increasing role for the kidney in removal of propylene glycol from the body. This supports the view expressed in the Draft Report (page 40, paragraph 3) that patients with impaired liver or kidney function may be at increased risk for developing propylene glycol toxicity. This would be the case, for example, with burns trauma patients undergoing silver sulfadiazine therapy, where compromised kidney function would be expected. However it should be appreciated that such individuals would be at risk of toxicity from any molecule present in the systemic circulation that was dependent upon renal (or hepatic) clearance in order to lower body burden. This includes drugs or vitamin preparations, which are, invariably, more toxic than propylene glycol.

2.5 Evidence presented in the Draft Report does not support the view that there exists a sub-population sensitive to propylene glycol: pre-existing disease states, coupled with concurrent administration of toxic drug therapies, explain the findings for many, if not all, of these studies.

The introductory sentence of this section (page 40, paragraph 3) correctly notes that "*Data on sensitive subpopulations are primarily associated with individuals with compromised liver or kidney function.*" This statement should be extended to note that it is rarely possible, in such cases, to disentangle the contributing role of the pre-existing disease state and concurrent administration of potentially toxic drug therapies on the overall clinical signs presented. The majority of these cases appear to suffer from hypoxia and/or liver damage suggesting an increased background level of lactate production (due to hypoxia) and a decreased capacity for the liver to convert lactate to pyruvate. The amount of lactate produced from the body mass due to the hypoxia would vastly overwhelm the amount provided from propylene glycol metabolism. In addition, if a pregnant woman suffered from one of these critical events, the hypoxia alone would be of much greater concern than any propylene glycol exposure. A decreased capacity of the liver to convert lactate to pyruvate to an extent where it would affect blood pH would be of concern without propylene glycol exposure simply due to the large amount of lactate naturally produced during pregnancy. Another point not considered in the review would be a competitive substrate inhibition between the metabolism of propylene glycol (*via* alcohol and aldehyde dehydrogenase) and the conversion of lactate to pyruvate. Both of these reactions require NAD^+ and in hepatocytes encountering increased lactate levels, a reduced amount of NAD^+ would be available to convert propylene glycol to lactaldehyde and lactate.

An additional issue when interpreting results from such studies is that information on increased osmolality, *etc*., is often obtained in a *post hoc* manner, *i.e*., there is no detail on whether the patient was exhibiting signs of incipient disease prior to whatever clinical crisis precipitated the case report, nor is there any way to tell if the patient would have shown altered osmolality or anion gap status, *etc.,* if some other pharmaceutical regime had been selected by the clinician. In other words, significant methodological and scientific controls are missing from these reports. The references presented in Table 2-8 are illustrative of the some of these confounders, for example:

- Fligner *et al.* (reference 29)
  Hyperosmolality in an 8-month old infant treated with silver sulfadiazine, a known renal toxicant and immunoactive agent, formulated with 7.67% propylene glycol. Drug-induced impairment of renal clearance therefore suspected, hepatic status not known.

- Huggon *et al.* (reference 62)
  An infant with inadequate cardiac output after open heart surgery was given a diuretic (containing 43% propylene glycol) to compensate for inadequate renal function. Increased serum hyperosmolality was noted in response to treatment. Pre-existing impairment of renal function seems likely in view of low cardiac output.

- MacDonald *et al.* (reference 98)
  An increased incidence of seizures was noted in 49 premature infants (<1.5 kg at birth) given megadoses of a multivitamin preparation (MVI-12, 10 ml/day, equivalent to 3 g propylene glycol/day), while a further 78 infants given an alternate therapy (MVI concentrate, 1 ml/d, equivalent to 300 mg propylene glycol/day) were seizure-free. These authors (Glasgow *et al.*, reference 30) had previously noted that the manufacturer of MVI-12 explicitly recommends that it should not be given to children under 11 years of age. Hypervitaminosis-linked toxicity seems probable.

- Glasgow *et al.* (reference 30)
  Increased osmol gap in 11 (predominately premature) infants following intravenous administration of mega-doses of a vitamin preparation (MVI-12, 10 ml/day, equivalent to 3 g propylene glycol/day) not recommended by the manufacturer for use in children less than 11 years old. Hypervitaminosis seems probable.

- Arulanantham *et al.* (reference 96)
  Seizures in an 11-year old boy given 2-4 mg dihydrotachysterol (vitamin D analogue) in 2-4 ml vehicle (98% propylene glycol) twice daily for 13 months. It is estimated that intake of propylene glycol would have been around 160-314 mg/kg bw/day assuming a 25 kg body weight. The presumptive identification of propylene glycol as sole causative agent is inconsistent with the absence of neurological effects in animals given 2,100 or 5,000 mg/kg bw/day (rat: Gaunt *et al.* (reference 72); dog: Weil *et al.* (reference 74).

- Yorgin *et al.* (reference 99)
  A 16-year old boy was admitted to hospital with seizures of increasing severity and given a variety of medications (including phenytoin, Lorazepam and barbiturates). His daily intake of propylene glycol from all sources was estimated to be 30-90 g/day. Serum creatinine increased after initiation of high-dose barbiturates (reported as up to13 g/day). Renal biopsy on day 14 showed proximal tubular swelling and vacuole formation. It is not possible to determine whether the exacerbation of symptoms seen in this patient was due to propylene glycol, the high-dose pharmaceutical regime used in his treatment or some other underlying disease process.

- Lolin *et al.* (reference 100)
  A 39-year old woman with a pre-existing history of uncontrollable epilepsy was admitted to the hospital with repetitive generalized convulsions. Blood analysis on admission revealed metabolic acidosis, hyperosmolality and the presence propylene glycol (4,000

mg/l) and ethanol (900 mg/l). Propylene glycol was reportedly present in fruit juice (0.06%), ethanol in mouthwash (30%), no other sources (including presence in medication) identified. Seizures subsequently ended when she stopped taking medication.

- Arbour (reference 63)
  A 45-year old man with pneumonia and septic shock was admitted to the hospital in a state of respiratory distress. He required sedation (Lorezapam, fentanyl) prior to tracheal intubation and positive ventilation (necessary to maintain vital signs). Dopamine hydrochloride was used to combat low blood pressure, while ceftriaxone, erythromycin and vancomycin were given for the infection. Therapy (including sedation) continued for a total of 38 days. Metabolic acidosis and a hyperosmolar state developed on day 30. The Lorezapam infusion contained 40% propylene glycol. The concentration of propylene glycol in serum was 1,700 mg/l.

- Cate and Hedrick (reference 60)
  A 58-year old man with pre-existing azotemic renal disease was admitted to hospital 'in a stupor'. GC analysis revealed propylene glycol was present at 700 mg/l in blood and 600 mg/l in urine, with elevated lactate and anion gap. The source of the propylene glycol responsible for the intoxication was not identified (*i.e.,* no medication involved). Poor renal clearance, due to pre-existing azotemic disease, probably exacerbated this case.

- Arbour and Esparis (reference 42)
  Osmolar gap metabolic acidosis in a 60-year old man with existing hypoxemic respiratory failure (predisposition to metabolic acidosis), which required heavy sedation (Lorezapam infusion, containing 83% propylene glycol) and positive-pressure ventilation for treatment. Compromised renal function seems probable, hepatic status unknown, osmol gap on admission (prior treatment) not determined.

- Bedichek and Kirschbaum (reference 101)
  A 70-year old woman was given diazepam, phenytoin, pentobarbital and etomidate to control post-operative seizures. She received 479 g propylene glycol by intravenous infusion over 24 hr. The etomidate treatment was 50% greater than the maximum dose recommended by the manufacturer.

The case studies cited in Table 2-8 cover a 22-year period between 1978 and 2000. They describe 69 cases where propylene glycol was considered to have played a contributing role to an effect. Examination of the original reports, however, shows that other, alternate explanations are available. In the case of the 60 infants described by MacDonald *et al.* (reference 98) and Glasgow *et al.* (reference 30), potential side effects linked to administration of megadoses of a vitamin preparation intended solely for older children have been ignored in the Draft Report. In the remaining 9 cases, confounding due to pre-existing disease and/or use of powerful pharmacologically active agents (including large and sustained doses of sedatives) probably has a contributing role.

While accurate information on the total number of patients (adults, children, infants) given pharmaceutical preparations containing propylene glycol during those 22 years is unknown, it can be conservatively estimated to be in the millions (perhaps even tens of millions

worldwide). It is reasonable to assume that a proportion of these cases will have pre-existing liver or kidney failure. This supports the view that propylene glycol poses little, if any, risk as an excipient in medicinal products and argues against the view that some sensitive subpopulation exists.

Finally, it is untrue to state (page 40, paragraph 4) that "*Propylene glycol toxicity can be suspected in patients having an abnormal serum osmolal gap.*" As correctly noted in the footnote on page 40 of the Draft Report, an increased osmolal gap is indicative of increased *solute* in blood, not simply or solely due to the presence of propylene glycol (any standard clinical text will provide further information on this point).

2.6 Species-specific differences in hemoglobin composition are likely responsible for red blood cell effects reported in the cat. The different structure of human hemoglobin indicates it would be less susceptible to propylene glycol-induced denaturation. These important species differences, which clearly limit any utility of these data for human risk assessment, should be highlighted in the revised Report.

The Draft Report concludes (page 43, first paragraph) that the domestic cat is the most sensitive species for propylene glycol toxicity, responding with Heinz body anemia when propylene glycol is present as an additive to the diet. (Note: reference 74 cited in this sentence refers to the beagle dog and should therefore be deleted).

While it is true that the cat does demonstrate species-specific sensitivity, this statement inaccurately reflects the findings obtained by Bauer et al. (reference 80) and Christopher et al. (reference 82). Bauer et al. described hematological changes in cats fed diets containing up to 12% propylene glycol, including Heinz body formation and decreased RBC survival. However, they reported 'only slight changes…in RBC counts' and did not describe it as anemia. Christopher et al. described hematological changes in cats fed diets containing up to 41% propylene glycol, including Heinz body formation and decreased RBC survival, but no anemia. In fact, Christopher et al. also described an increased production of RBC, which precluded any anemia in the cats, despite decreased RBC survival.

Another study conducted in cats, but not mentioned in the Draft Report, identified an increase in Heinz bodies following dietary administration of propylene glycol over 2-3 months (Quast et al., 1979; see attached robust summary). Increased hemosiderin deposits were also noted in liver and spleen, but appeared secondary to Heinz body formation. The NOAEL for Heinz body formation was 80 mg/kg bw/day, with a LOAEL of 443 mg/kg bw/day. No other systemic effects were seen in cats at doses up to 4,239 mg/kg bw/day (the maximum used in the investigation), including no evidence of hemolytic anemia. (A Robust Summary providing further information on this study is appended as Attachment C).

Thus, a more precise description of the species-specific effects of propylene glycol in cats would be that cats demonstrate Heinz body formation and decreased erythrocyte survival when propylene glycol is present as an additive (6% w/w or above) to the diet.

At the request of the PO/PG Panel, Dr. Mark Udden, an expert clinical hematologist based at Baylor College of Medicine (Houston, Texas) evaluated these studies and their relevance to humans. A copy of Dr. Udden's report is attached.

Dr. Udden notes that cat hemoglobin contains more reactive SH groups than hemoglobin from other mammals, making it highly sensitive to oxidant-induced denaturation. Since propylene glycol itself does not directly induce Heinz bodies following incubation with cat erythrocytes *in vitro*, it appears that a metabolite is responsible for this effect. Metabolic considerations indicates that propylene glycol is converted to D-lactate *via* a GSH-dependent process *in vivo*, indicating that glutathione depletion is probable in cats ingesting large amounts of this material. High doses of propylene glycol are also reported to alter the NAD/NADH ratio in red cells, leading to an altered redox state. Removal of damaged red blood cells from the circulation is also less efficient in the cat compared to other species, due to differences in the structure and function of the spleen.

Overall, oxidant-induced denaturation of SH groups present in cat hemoglobin would be enhanced under such conditions, leading to the Heinz body formation. Inefficient removal of damaged red cells from the circulation by the spleen would further accentuate the magnitude of the response induced in the cat. The differential structure of human hemoglobin indicates it would be less susceptible to propylene glycol-induced Heinz body formation, while any cells that were affected would be removed efficiently by the spleen. These important species differences, which clearly limit the utility of the cat data for human risk assessment, should be highlighted in the revised Draft Report.

Finally, for the reasons discussed above, the Draft Report should be revised to note that while the data of Bauer *et al.* and Christopher *et al.* are indicative of hemoglobin/erythrocyte damage, they do <u>not</u> support an "impairment of hematopoiesis" (Expert Panel conclusion, page 34, paragraph 4). In fact the converse appears to be the case since anemia is absent or mild in the cat indicating <u>*increased*</u> formation of red cells by the bone marrow.

2.7 Interpretations of hematological findings from several of the animal studies cited in the Draft Report contains errors and inconsistencies that should be addressed before the report is finalized. Overall, evidence that propylene glycol adversely affects the erythrocyte in rats and monkeys is lacking.

As part of the evaluation of propylene glycol, the Draft Report concludes (page 45, first paragraph) that intermediate and chronic exposure to propylene glycol may result in changes in hematological parameters and hemolysis of red blood cells in animals, with limited evidence of similar effects in humans. Review of the individual references cited in the Draft Report to support this view demonstrates some inconsistencies with the original study findings that should be addressed before the report is finalized. These include:

- <u>Suber *et al.*</u> (reference 76)
  The Draft Report notes (page 33, paragraph 3) that female rats exposed to 707 ppm propylene glycol aerosol for 90-days exhibited a decrease in hemoglobin concentration, while no dose-related changes in red blood cells were observed in males. This appears to relate to a 2.8% reduction in mean corpuscular hemoglobin noted in these animals. Although statistically significant, the small magnitude of the effect coupled with an absence of any dose-response relationship suggests this was a chance event of doubtful biological relevance.

- Robertson *et al.* (reference 77)
  The Draft Report correctly states (page 33, paragraph 3) that hemoglobin counts increased in Rhesus monkeys following 13 months inhalation exposure to propylene glycol. It should also be noted, however, that "*the monkeys were all found to be suffering from a considerable degree of anemia*" upon delivery to the laboratory at the start of the study, and that hemoglobin counts were increased also in the *controls* at 13 months. Indeed, *every* hematological parameter improved over this time, an occurrence the authors linked to the better nutritional status of the animals after receipt by the laboratory.

- Saini *et al.* (reference 81)
  The Draft Report concludes (page 33, paragraph 3) that these results demonstrate that hemoglobin, packed cell volume and red cell counts were decreased significantly in rats following a single oral treatment with propylene glycol. Inspection of the Saini *et al.* study report reveals, quite surprisingly, that no control group was included in this study. Such a fundamental flaw in experimental design (plus the fact that no red cell effects were reported by Gaunt *et al.* in rats receiving comparable oral exposures) raises doubts about the reliability of these observations, and suggests they should be excluded from the CERHR review.

Overall, results from these studies do *not* support the view expressed in the Draft Report that propylene glycol adversely affects the erythrocyte in rats and monkeys. The Draft Report should be revised to reflect the inconsistencies and uncertainties noted above.

2.8 The mechanistic basis of hematological changes seen in humans should be presented more clearly in the Draft Report.

The Draft Report concludes (page 45, first paragraph) that there are "limited substantiated data" to indicate that propylene glycol damages red cells in humans. This statement is based upon case reports of patients receiving propylene glycol during intravenous drug therapy.

As noted by Dr. Udden, hemolysis occurs in such cases because the erythrocyte membrane is permeable to propylene glycol, which enters the cell along with an equivalent amount of water. Experimental studies demonstrate that propylene glycol concentrations in excess of 30% are necessary to produce this damage in human erythrocytes, which arises as a consequence of an osmotic imbalance within the cells. This is distinct from the mechanism operative in the cat, where the hemoglobin is susceptible to pro-oxidant damage due to the presence of reactive SH groups.

This fundamental difference in erythrocyte response to propylene glycol between humans and cats requires clear explanation in the report.

2.9 The evaluation of human and animal skin irritation data in the Draft Report is superficial and inaccurate.

As noted in the Draft Report (page 25, paragraph 1), local skin reactions in humans following topical application of propylene glycol (either neat or in combination with other (cosmetic) ingredients) have been the subject of some debate. This reflects divergent study designs, inconsistent interpretive criteria and fundamental differences in the nature of the test populations that have been studied (*i.e.*, healthy volunteers, atopic individuals, allergic/eczema

cases) by different investigators. Even with normal healthy subjects, the incidence of apparently 'irritant' responses to undiluted propylene glycol is highly variable, and may be as low as 0% (0/203 volunteers) or as high as 40% (14/35) (reviewed by Funk and Maibach, 1994). Atopic or hypersensitive individuals may respond to propylene glycol in a manner consistent with sensitization (Funk and Maibach, 1994), although there appears to be little consistency in the incidence or magnitude of the reactivity. As noted in the Draft Report, factors such as temperature and humidity at the time of exposure affect the response of normal human skin, perhaps influencing dermal hydration and integrity of the barrier function of the skin. Negative results when propylene glycol was tested in the local lymph node assay (Basketter *et al.*, 2000) and the mouse ear swelling test (Descotes *et al.*, 1988) also tend to rule out an immunological basis for the reactions reported in these individuals. Overall it appears that undiluted propylene glycol has the potential to produce at most minimal irritation in a small fraction of human subjects although responses in atopic or hypersensitive individuals may be less readily quantifiable.

As noted subsequently in the Draft Report (page 31, final paragraph), propylene glycol has also been tested on rabbit skin, following standard protocols. This includes results obtained using OECD Guideline 404, an internationally-agreed 'gold standard' used for assessing the skin irritation potential of chemical substances. Results from these studies (which use a well defined, standardized methodology) are helpful when attempting to unravel the relevance of skin reactions in humans (often obtained using non-standard, less well defined methods). Therefore the statement (page 32, paragraph 1) that "*It is of dubious relevance to have negative results in rabbits regarding [skin] irritation when the irritation potential, although minimal, of propylene glycol has been established in man*" suggests that the CERHR is unaware of the prevailing international acceptance of the OECD 404 method as well as the uncertainty and inconsistencies present in the human database.

These sections of the report should be re-written to reflect more precisely the strengths and weaknesses present in the human and animal database.

2.10 Certain statements and references used in the Draft Report to describe the toxicokinetics of propylene glycol in humans and animals are unclear or unreliable, and should be removed or amended in the final report.

Section 2.1.1 Absorption

Human, oral

- Page 8, paragraphs 3 and 4: The kinetic constants obtained by Yu *et al.* (1985; reference 23) are unreliable since subjects were given significant amounts of ethanol in addition to propylene glycol. Both substances are substrates for alcohol dehydrogenase, hence competitive inhibition of metabolism would have occurred.
- Page 8, paragraph 4: Under Strength/Weaknesses section: doses described as being '…above metabolic saturation…', but no supporting evidence for this is offered. Indeed, the same paragraphs reiterate the 'rapid absorption, distribution into total body water, relatively short half-life, and rapid total body clearance,' all of which would contradict saturation kinetics being important. Also, if the volume of distribution indicates that propylene glycol is distributed in total body water, bioavailability must be high, so it is

not clear why the report states that data are not adequate to judge bioavailability. There may not be data to calculate exact bioavailability, but certainly one can 'judge' or assume high bioavailability from these data.

- Page 9, paragraph 2: Strength/Weaknesses section incorrectly states that "*it appears that children absorb propylene glycol significantly faster and attain higher peak plasma concentrations than adults…*" A simple calculation to compare administered dose to plasma maximum concentration values shows that this is not accurate. Dose to the child was higher, so it is expected that maximum concentrations would be higher. Actually administered doses and maximum concentration values were almost perfectly proportional:

  child/adult dose ratio =173/123 = 1.39 ~ maximum concentration ratio of 2.2/1.6 = 1.41

Human, dermal

- Page 9, paragraph 3: Quantitative data from burns trauma patients obtained by Kulick *et al.* (1985; reference 28) and Fligner *et al.* (1985; reference 29) are unreliable: Sulfadiazine, the antimicrobial treatment used to treat these cases, is an established renal toxicant. Hypovolemic shock also results in altered renal function and plasma osmolality independent of clinical intervention. Metabolism and clearance of propylene glycol in these individuals would therefore have been compromised.
- Page 9, paragraph 3: The case study by Glasgow *et al.* (1983; reference 30) contains no evidence to support the statement that a blood propylene glycol level of 700 mg/l was due to an anti-fungal ointment used to treat diaper rash: this is an unsubstantiated opinion.

Section 2.1.3 Metabolism

- Page 12, paragraph 4: Yu *et al.* (reference 23) should be changed to Yu and Sawchuck (reference 44).
- Page 12: Contradiction for renal clearance versus metabolic clearance. The Draft Report cites several datasets for urinary disposition or renal clearance of propylene glycol, including the following:
  - dog: 55-88% of dose
  - human: 45% of dose
  - rabbit: 2.4-14.2% of dose
  - rat: 2-17% of dose

  Given these data, it is difficult to understand how the Draft Report states that '*Metabolic clearance accounts for 85.8-97.6% of total clearance*' as separate from renal clearance.
- Page 13, paragraph 2: The clinical parameters cited from the study by Arbour and Esparis (2000; reference 42) were obtained from a highly sedated patient undergoing forced ventilation to treat pre-existing hypoxemic respiratory failure. Given the abnormal physiological condition of the subject, it is doubtful that these values are reliable or representative.

- Page 13, paragraph 2: Infants (including a significant number of premature births) from the study by Glasgow *et al.* (1983; reference 30) had received an excessive amount of a multi-vitamin preparation intended for older children (age 11 and above). Toxic hypervitaminosis, with probable impairment of hepatic and renal function, would be expected under such conditions. This study is, therefore, unreliable and does *not* provide credible evidence that the half-life of propylene glycol is prolonged in normal infants.

- Page 15: Strength/Weaknesses: Several inaccurate statements and inconsistencies in this section, including the following:

  Accumulation of D-lactate was demonstrated only in cats, not in rabbits (reference 34 is a rabbit study); in fact, rabbit data demonstrated L-lactatemia, not D-lactatemia.

  No reference provided to support statement for D-lactate accumulation in humans; in fact there is a study described (Oh *et al.*, reference 48) that demonstrates '*...D-lactate was shown to be efficiently utilized in man (reference 48).*' (p. 16).

  No data provided to support conclusion that D-propylene glycol clearance would be slow in humans; contradictory results between cat and rabbit do not indicate which is a more probable model for humans. Cat-specific effects (Heinz body formation) indicate cats are not a good model for humans.

- Page 19: Utility: The statement that the cat study reported by Christopher *et al.* (reference 33) is useful to link human data (reference 48; Oh *et al*., 1985) with animal data, is not correct. Oh *et al.* (reference 48) describes D-lactate blood levels and excretion in human volunteers following infusion of multi-gram doses of D,L-lactate. Increases in dose/infusion rate resulted in increased urinary excretion, but no change in overall clearance, and no D-lactatemia.

  Overall summary: more precise language should be used: lactatemia is not the same physiological entity as lactate acidosis. D-lactate does not seem to accumulate in humans, based on Oh *et al.* (1985), as it does in cats. Therefore, the cat may not be a useful model for propylene glycol PK/metabolism in humans.

- Page 20, paragraph 2: The sentence "*Sjoblom, et al. (51) found that in Wistar rats ADH activity in liver was not detected at birth, was 3% of adult activity on postnatal day 20, and continued to increase with age to 65% and 82% of adult activity on postnatal day 21 and 47, respectively*" suggests that liver ADH activity increases from 3% of adult activity to 65% of adult activity overnight (from postnatal day 20 to postnatal day 21). The 3% of adult activity is probably on gestation day 20, not postnatal day 20.

  On page 20 of the Draft Report, under the section titled "Utility (adequacy) for CERHR evaluation process," the reviewers engage in some speculation regarding the proximate toxicant for propylene glycol. Included in the speculation is that the fetus would be adversely affected from lactate produced from propylene glycol metabolism, that any maternal acidosis would also cause acidosis in the fetus, and that due to the lack of metabolism of propylene glycol by the fetus/newborn/infant, more severe CNS effects from propylene glycol may become manifest.

It is important to remember that propylene glycol has not been demonstrated to cause any developmental toxicity in any laboratory species or humans to date, even at doses exceeding 10,000 mg/kg bw/day. Therefore, the need to identify a "proximate toxicant" is of questionable value since there is no toxicity for the "proximate toxicant" to identify with. Any concerns regarding D- or L-lactate affecting development should be alleviated with this information.

- Page 20, paragraph 3: The Draft Report should include an explanation of the reasoning behind the acceptance of the data from the Pikkarainen and Raiha (reference 26) paper over the data from the Smith *et al*. (reference 52) paper. The Pikkarainen and Raiha paper suggests that total ADH activity in human infants was 20% that of adults while the Smith *et al*. paper (which, in addition, followed isoform changes in ADH activity) reported activity in children less than one year old as 50% that of adults. Under Strengths/Weaknesses" on that same page, the Pikkarainen and Raiha data is discussed but the Smith data is not. In addition, later in the chapter (page 22), the data from Pikkarainen and Raiha is reiterated again, without any discussion of the Smith *et al*. data. In addition, the "Utility" section on page 20 seems to engage to unsupported speculation of whether or not embryos/fetuses experience metabolic acidosis similar to those present in the mother as well as speculation on the CNS toxicity of propylene glycol itself in newborns and infants. Substantiating these claims with actual data is necessary before these hypotheses are accepted.
- Page 44, paragraph 2 (Section 2.6 – Summary): The statement '*Renal excretion is a small percentage of the dose…*' is not correct, considering the earlier statement that 45% of propylene glycol is cleared in humans *via* urinary excretion

Section 2.1.4 Elimination

- Page 21, paragraph 5: The kinetic constants obtained by Yu *et al.* (1985; reference 23) are questionable since subjects were given significant amounts of ethanol in addition to propylene glycol. Both substances are substrates for alcohol dehydrogenase, hence competitive inhibition of metabolism would have occurred.
- Page 22, paragraph 1: Elimination of propylene glycol by the infant described by Fligner *et al.* (1985; reference 29) would have been impaired due to concurrent treatment with silver sulfadiazine (renal toxicant). It is highly likely that hypovolemic shock would have also resulted in altered renal function and plasma osmolality. The 11 infants (including a significant number of premature births) included in the study by Glasgow *et al.* (1983; reference 30) had received an excessive amount of a multi-vitamin preparation intended for older children (age 11 and above). Toxic hypervitaminosis, with probable impairment of hepatic and renal function, therefore undermines the reliability of these findings.
- Page 22, paragraph 3: The elimination half-life obtained by Yu *et al.* (1985; reference 23) is unreliable due to concurrent administration of ethanol, a competitive inhibitor of propylene glycol metabolism.
- Page 22, paragraph 4: The prolonged half-life for infants noted by Fligner *et al.* (1985; reference 29) was most likely a consequence of hypovolemic shock and sulfadiazine-

induced renal failure. The longer half-life derived from Glasgow *et al*. (1983; reference 30) in a group that included a number of premature births occurred subsequent to over-administration of a multi-vitamin preparation; toxic hypervitaminosis, with impact on liver and kidney function, therefore seem probable.

- Page 22, paragraph 5 (and Page 45, paragraph 6): The half-lives of propylene glycol in infants is stated as "10.8 to 30.5 hours" from references 29 and 30. These half-lives are then compared to the adult values and reference is made to the Pikkarainen and Raiha paper once again. However, in the paragraph just above, the report states that with increasing dose, the half-lives in adults change from a first-order process to a zero order process "at or above a dose of 5.1 g/day." The report does not attempt to interpret the dose level the infants received in terms of half-life for elimination. For comparison, the blood levels of propylene glycol in the adult studies ranged from 0.63 to 5.6 mM, while the blood levels in the infants ranged from 8.5 to 139 mM. Such differences in dosages would be expected to affect the elimination processes and thereby affect the half-lives (first-order vs. zero-order), as was described in the Draft Report. It is difficult to discern the difference in elimination rate constants due to age and developmental stage with such a large disparity in dosage (as indicated by blood concentrations). This problem in interpretation is repeated in the section on "Potentially Sensitive Subpopulations" on page 45. Again, the summary of the Page 22 conclusion is repeated with no caveats (of which there should be several).
- Page 23, paragraph 4: The Draft Report's suggestion of construction of a PBPK model for propylene glycol is questioned. Given the wealth of actual human data, it is unclear what use a model laden with assumptions will have. The suggestion appears purely academic and not relevant to the review and current risk assessment, and hence should be stricken from the report.

2.11 Certain statements and references used in the Draft Report to describe the general toxicity of propylene glycol are unclear or unreliable and should be amended or removed from the final report.

General toxicity

- Page 24, paragraph 2: While the meaning of sections of this paragraph are unclear (for instance, what is meant by "*There are few apparent weaknesses like the statement that impaired renal function increases toxicity of propylene glycol*" ?), the overall conclusion appears to be that decreased renal clearance will have little impact on the potential toxicity of propylene glycol. This appears a simplistic view of the role of the kidney in homeostasis, which ignores the fact that normal renal function is essential for the excretion and/or re-absorption of a range of endogenous (*e.g*., lactate, see Oh *et al*. (1985, reference 48) and exogenous (*e.g*., pharmaceuticals, propylene glycol) substances.

2.2.1 Humans

Oral Exposure

- Page 24, paragraph 4: The statement that "serum levels of >0.18 mg/l have resulted in toxicity" (Arbour and Esparis (2000), reference 42) is incorrect. The source paper

actually states that serum levels in excess of 18 mg/dl can be toxic (that is, 180 mg/l), however the authors provide no analytical or literature support for this assertion. In the absence of scientific support, this comment should be deleted from the Draft Report. (It also occurs on page 12, paragraph 3).

- Page 24, paragraph 4: Seizures and CNS depression are not unsurprising in a child apparently receiving 4-8 g vitamin D per day for 13 months (citation from LaKind *et al.,* 1999, reference 22). Under such circumstances it appears unlikely that propylene glycol could be the sole causative agent.
- Page 24, paragraph 4: References 63 and 42 (Arbour (1999) and Arbour (2000), respectively) contain no data to support the statement that "*Chronic ingestion of propylene glycol has resulted in lactic acidosis, stupor and seizures in adults*." These references, in fact, consider the side effects of short-term intravenous infusion of Lorazepam (containing propylene glycol as an excipient) used during positive pressure ventilation needed to treat hypoxemic respiratory failure.
- Page 44, paragraph 4 (Section 2.6 – Summary): The mortality mentioned for infants comes from compromised infants; there are no mortality data reported for any healthy infants or for adults.
- Page 45, paragraph 1 (Section 2.6 – Summary): There are contradictory data for any effect of propylene glycol on hemoglobin values; both increases and decreases cannot be indications of the same effect, hemolysis. Again, 5 gram/kg bw/day is 5-fold higher than what current testing guidelines allow for maximum dose (40 CFR 798.2650), and this should be noted. No data, let alone any substantiated data, were described to indicate any hemolysis effect in humans. Such a statement needs references, which should be described in the body of the report.

2.2.2 Experimental Animal Data

Oral Exposure

- Page 28, paragraph 3: The NOAEL values given in the final sentence of this paragraph are reversed (*i.e.* Gaunt *et al.* reported that 1.7 g/kg bw was the value for males and 2.1 g/kg bw for females).
- Page 28, last paragraph: Exception is taken with the comment "*that a higher dose was unfortunately not used.*" The high dose in this chronic study exceeded current guidelines on the limit of high doses that need be administered in such studies (1,000 mg/kg bw; (40 CFR 798.2650) and is fully informative that such a dose is not chronically toxic. Pushing doses to extremes for the purposes of demonstrating toxicity produces results that are difficult to interpret relative to reasonable exposures and causes unnecessary pain and suffering in animals.

  Regulatory agencies recognize this problem, and accept it is inappropriate to subject animals to ever-increasing exposures simply to see if there is an effect. EPA, for example, notes in its oral toxicity test guideline (40 CFR 798.2650) that treatment of rats and mice with doses in excess of 1,000 mg/kg bw/day may be unnecessary if no adverse effects are seen at that treatment level. Other guidelines (40 CFR 798.3260, chronic

toxicity; 40 CFR 798.3300, oncogenicity) place an upper limit of 5% on the maximum amount of a test substance that should be added to diet in a feed incorporation study.

- Page 29, paragraph 3: Exception is taken to the high regard afforded the 1931 Seidenfeld and Hanzlik study. The limitations of this report are not presented and instead the Draft Report remarks only on its enjoyable reading and the presence of findings in the liver. Although possibly supporting the vaguely reported liver findings in the Morris report, these findings are not consistent with the more modern and robustly designed chronic study of Gaunt *et al.* (1972; reference 72) that did not report histopatholgy changes to the liver at similar dosages, making the early reports of such findings suspect.
- Page 33 and 34: The Draft Report attempts to draw conclusions vis-à-vis hemolysis based on reported hemoglobin (Hgb) values from several studies in different species. However, there are contradictory results reported for hemoglobin values: dog and female rat showed decreased Hgb, while monkeys showed increased Hgb and male rat showed no change. Nonetheless, the conclusions in the Draft Report are that all the Hgb changes are consistent with hemolysis. Both increases and decreases cannot be associated with hemolysis!
- Page 33, paragraph 7: Utility: The statement about *in vitro* hemolytic capability of propylene glycol needs to be qualified by the simple fact that high doses of propylene glycol, especially *in vitro*, will change osmolarity of culture medium, and thus affect hemolysis.
- Page 33, paragraph 8: Strength/Weaknesses: The statement that changes were overcome by adaptation during chronic exposure fails to take into account the importance of route of administration. Actually it is more probable that oral gavage versus dietary administration (*i.e.*, rapid bolus versus slower ingestion) accounted for the effects noted in the acute and lack of effects in the chronic study, rather than adaptation.

Genetic Toxicity

2.3.2 Experimental Systems

Page 36, paragraph 6: Osmolarity was an issue for the positive dose in the reference cited (86). At 32 mg/ml, equivalent to 30 mOsM, the results are of questionable biological significance.

2.4 Carcinogenicity

- Page 39, paragraph 2: The criticism of the number of carcinogenicity studies available for propylene glycol in unwarranted. The quality of available information is more important to the assessment than the quantity of studies. Carcinogenicity studies are animal intensive and hence undertaking more studies in the face of the very low toxicity demonstrated in the available repeated exposure studies and from human experience, is an inappropriate use of animal resources.
- Page 40, paragraph 3: The view expressed at this point in the Draft Report appears inconsistent with the criticism regarding 'the limited number of studies' expressed on page 39. The Draft Report appears to suggest here that the study was unnecessary as the result

was predictable, whereas was previously critical that too few cancer studies having been performed. In any case, although the result may have been predicted, for chemical substances such as propylene glycol that have the potential for significant dermal exposure, it would seem prudent to test the chemical and demonstrate its safety.

2.5 Potentially Sensitive Subpopulations

- Page 40, paragraph 5: Oral and Intravenous Use section: no information provided to support description of 'individuals with a chemical sensitivity to propylene glycol'.

3. Existing occupational and consumer product information should be sufficient to conduct a screening level exposure assessment and an evaluation of risk for the demonstrated very low hazard substance propylene glycol.

3.1 Per capita daily intake of propylene glycol data for the United States are available.

The last sentence of paragraph 1 of Section 1.2.4 states "No data were available for average daily intake in the United States." Such data are available. In its recent update of the use of propylene glycol and related compounds as flavoring agents in food, the United Nations Joint FAO/WHO Expert Committee on Food Additives (JECFA) states that, for food uses of aliphatic acyclic diols, triols, and related substances (JECFA, 2002):

> In the USA, three substances, namely glycerol (220,000 μg/day), triacetin (83,000 μg/day), and propylene glycol (2,400,000 μg/day) accounted for 96% of the total annual daily *per capita* intake. [p. 347].

Calculation of the 2,400,000 μg/day (2.4 g/day) *per capita* daily intake of propylene glycol in food in the United States is based on the 1995 update of data collected since 1972 by the Flavor and Extract Manufacturers' Association (Lucas *et al.*, 1999).

3.2 Characterizing propylene glycol, a food additive, as a "contaminant" in consumer products is inaccurate and inappropriate

Paragraph 2 of Section 1.2.4 of the Draft Report inappropriately characterizes propylene glycol as a potential "contaminant." As stated in Section 1.2.3, propylene glycol is rapidly degraded in water and has not been reported to occur in drinking water or groundwater. Further, as stated in Section 1.2.2, propylene glycol is a GRAS chemical, approved for use by the FDA in food for humans and all animal species but cats, and is also used in personal care products such as cosmetics and toiletries to provide desired characteristics. Rather than "drinking, bathing in, or showering with [propylene glycol] *contaminated* water," (emphasis added), the world's population routinely eats, drinks, bathes in and showers with products purposely formulated to include propylene glycol because of its long history of widespread use, supported and recognized in the FDA's GRAS designation and in JECFA's (2002) conclusions. Propylene glycol has also long been purposely used globally as a pharmaceutical excipient to help deliver life-saving and life-enhancing drugs.

3.3 Human exposure data should be sufficient to reach conclusions about risk

The summary of human exposure data presented in the Draft Report is inadequate because, given the documented high levels of human exposure through dietary intake in the United States (JECFA, 2002), the quantitative occupational exposure studies discussed (or that should be discussed, see detailed comments in Section 4) in the Draft Report, and the long history of worldwide use of propylene glycol in food, prescription and non-prescription drug products, cosmetics, toiletries, soaps, and personal care products of many varieties, a screening level exposure assessment is all that is needed to draw risk conclusions. Propylene glycol is used in consumer products throughout the world and has a long history of widespread use. Yet there have been only a very few reports of adverse effects, some of which are likely unrelated to propylene glycol exposures. The history and pattern of propylene glycol use supports the emprirical conclusion that exposure to propylene glycol poses a very low risk to humans. An evaluation of risks associated with exposures to propylene glycol would not be complete without simultaneous consideration of the history of beneficial uses of propylene glycol as a pharmaceutical excipient, helping to deliver life-saving and life-enhancing drugs to very sick people.

4. Specific Comments on Chemistry, Use and Human Exposure

Section 1.1.1 Nomenclature

- Sirlene is a trade name no longer used.
- PG12 and Solar Winter Ban are terms not common in the industry.

Section 1.1.3 Chemical and Physical Properties

- "Tasteless" is not an appropriate description for propylene glycol. Its taste has been described as “sweet and bitter.”
- Table 1-1
  - Melting point is not an appropriate terminology for propylene glycol, which supercools at <-60 ºC.
  - What is reported as “Specific Gravity” is actually “Density.”

Section 1.1.4 Technical Products and Impurities

The major impurities in propylene glycol are water (<0.2%) and dipropylene glycol (<0.2%). Chlorides and iron are sometimes present at 1 ppm or less. Heavy metals, arsenic and sulfates are not found in propylene glycol manufactured with modern technology. The materials and concentrations listed as “impurities” in this section are simply specifications set by the USP, anachronisms from the original USP monographs, and should not even be mentioned here.

PG-12 and Sirlene are no longer used as trade names and should be deleted.

Current manufacturers of propylene glycol in North America are The Dow Chemical Company in Freeport, TX, and Plaquemine, LA; Lyondell Chemical Company in Pasadena, TX; Huntsman Corporation in Port Neches, TX; and Arch Chemicals, Inc., in Brandenburg, KY.

Section 1.2.1 Production Information

Paragraph 3 of Section 1.2.1 should be replaced with information about the percent of production used in various applications reported in the SIDS Initial Assessment Report (reference 4), as follows:

USES OF PROPYLENE GLYCOL

USES	APPLICATION	**FUNCTION**	**% PRODUCTION** (1)	(2)
Intermediate	Unsaturated Polyester Resins	Resin Monomer	38%	40%
Substance Substance	Food, Pharmaceuticals Cosmetics and Personal Care	Humectant Emollient	17%	12%
Substance	Specialty Antifreeze, Aircraft Deicing, Industrial Lubricants, Inks	Lubricant, Coolant	13%	10%
Substance	Liquid Laundry Detergents	Dispersant	9%	15%
Substance	Pet Foods	Humectant	5%	6%
Substance	Tobacco Processing	Humectant	4%	3%
Substance	Paints and Coatings	Solvent	4%	4%
Substance Intermediate	Miscellaneous	Miscellaneous Plasticizer	10%	4%

(Sources: Kirk-Othmer Encyclopedia of Chemical Technology, 3rd Ed., Volumes 1-26, NY, John Wiley and Sons 1978-1984; (1): Chemical Business, Nov. 1992, p. 36; (2): and Chemical Marketing Reporter, Vol 249, No. 7 p. 37, 2/12/96).

Section 1.2.2 Use

The first sentence of paragraph 5 should be modified to read as follows: "PG is used as a humectant in pet food products, but is not used in cat foods."

Section 1.2.4 Human Exposure

General Population Exposure

It is not clear what purpose is served by the comparison between propylene glycol and triethylene glycol in the last sentence, paragraph 4 (page 4) and Table 1-2. These and all other references to triethylene glycol should be deleted.

The information contained in paragraph 5 should be reconsidered in light of the information published in JECFA (2002) to provide a coherent discussion of *per capita* daily dietary intake. It seems possible that FDA's exposure estimate was based on data collected by the Flavors and Extracts Manufacturers' Association (Lucas *et al.*, 1999).

The discussion of general population exposure should also include information available in the Cosmetic Ingredient Review (CIR, 1994) for propylene glycol.

The Physician's Desk Reference also contains specific information about propylene glycol use as a pharmaceutical excipient.

Occupational Exposure

The correct reference for the NOES study is NIOSH (1983), included in the reference list at the end of this document. The Draft Report cites the NOES survey, conducted in the early 1980s, as the source of the estimate "*that 1,748,454 people were potentially exposed to propylene glycol at the workplace.*" Estimates derived from the NOES survey are of questionable value, and EPA (64 Fed. Reg. 46771 (Aug. 26, 1999)) has said, "*Now over 15 years*

old, the NOES data have become progressively dated, and as a consequence, less representative of current exposure situations."

The discussion of the Laitinen *et al.* (reference 16) study in paragraph 4 summarizes the conclusions of the study, which appear to all be about ethylene glycol and therefore are not appropriate to a discussion of propylene glycol.

Table 1-3 should be deleted since none of the products listed are currently made or marketed. The table should be replaced with a general statement such as,

> Propylene glycol is commonly used in aircraft de-icing fluids. Commercial Type I fluids containing propylene glycol are typically formulated with propylene glycol concentrations of approximately 88%, and Type IV fluids containing propylene glycol are typically formulated with propylene glycol concentrations at approximately 50%. Type I fluids are usually diluted with water before use, and Type IV fluids are frequently used in neat form although they may also be diluted with water for use.

The discussion of occupational exposure should contain some discussion of the use of propylene glycol as well as other glycols and mineral oils in the entertainment industry to generate artificial smoke and fog ("theatrical fogs"), an exposure which has been studied by NIOSH (1994) and Moline *et al.* (2000*)*. Members of the PO/PG Panel, a consortium of the major domestic propylene glycol producers and authors of these comments, have individually taken positions against this use of propylene glycol because of potential eye and upper respiratory tract irritation.

The discussion of occupational exposure should contain some discussion of the study of workplace exposure to water-based paints reported by Norbäck *et al.* (1995).

Section 1.4 Summary of Human Exposure Data

The first paragraph should match with the uses listed in Section 1.2.1.

As noted above in comments on Section 1.2.4, the statement in paragraph 3 that "No data were available for the US average daily intake of propylene glycol from food products or food packaging" is incorrect. Paragraph 3 should therefore be either removed entirely or substantially revised.

Paragraphs 5 and 6 inadequately represent the available data on occupational exposure to propylene glycol. The discussion of the Laitinen *et al.* (reference 16) study focuses on conclusions related to ethylene glycol. For propylene glycol, Laitinen *et al.* found no differences between controls and propylene glycol-exposed workers.

However, the summary of human exposure data should primarily be judged inadequate because, given the documented high levels of human exposure through dietary intake in the United States (JECFA, 2002), the quantitative occupational exposure studies discussed (or that should be discussed) in the Draft Report, and the long history of worldwide use of propylene glycol in food, prescription and non-prescription drug products, cosmetics, toiletries, soaps, and personal care products of many varieties, a screening level exposure assessment is all that is needed to draw risk conclusions. Propylene glycol is widely used throughout the world and has a long history of widespread use. Yet there have been only a very few reports of adverse effects,

some of which are arguably not related to propylene glycol exposures. The history and pattern of propylene glycol use supports, at least qualitatively, the conclusion that exposure to propylene glycol poses a very low risk to humans.

5. Specific Comments and Editorial Suggestions

N.B. The current Draft Report contains many typographical and grammatical errors that should be corrected. Careful proof-reading of the Final Report is required.

Page 5, Paragraph 2

The conclusion reached by Weislander *et al.* (reference 17) included the modifier "high exposures," which should be added to this discussion.

Page 6, Table 1-4

Is this total mg/m^3 for 6-hr collection or mean mg/m^3 based on 6-hr collection?

Pages 13, 28, 29, 30 and other instances

Repeatedly in this document, inappropriate comments and/or statements are made to the effect that if only the dose of propylene glycol had been higher, perhaps something would have happened, *i.e*., implying a toxic effect of some kind would have been noted. Likewise, references to the 'low doses used' reoccur, although actually these refer to doses at or above 2,000 mg/kg bw. Current international testing guidelines clearly delimit 1,000 mg/kg bw as a maximum recommended dose for testing, and consider 5% (50,000 ppm) as a maximum amount recommended in the diet, based on concerns for nutritional imbalance. Given these internationally accepted recommendations on test material administration, it should be recognized that propylene glycol is of low toxicity, and that doses of 2,000 mg/kg bw (2 grams/kg bw) should NOT be described as 'low doses.' In the same vein, effects noted at 8% and 10% of diet and higher, should be qualified as outside the range of current testing guidelines for test material dose, and the results viewed accordingly.

Page 24, Section 2.2.1

Correct units for statement on adult serum levels of propylene glycol: should read 0.18 mg/dL, or 1.8 mg/L.

Page 25

Weislander *et al.* (2001) measured chamber concentrations of up to 815 mg/m^3; consequently their results apply to very high exposure levels.

Page 31

Table 2-4: Clark *et al.* (reference 78) dose and NOEL is described as 0.52 g/kg bw; while the units are not correct (0.5 ml on rabbit skin for one rabbit would likely be about 0.52 g/~3 kg rabbit ~0.17 g/kg bw), it is misleading to report the dose in this manner, as this was a dermal irritation test, which employed neat material. It is not possible to test a higher amount than neat material. The most appropriate designation would be 'neat material not irritating,' rather than the current 'NOAEL 0.52 one time.'

Page 41

Comments on labeling requirements are not germane to the propylene glycol toxicity discussion, and the information provided in the last paragraph on page 41 characterizing requirements for listing inactive ingredients in drug labels cites a 1985 source of information that is not current. GRAS status has no bearing on why an inactive ingredient is or is not required to be listed on a drug label. "Dispensed" (*i.e.*, prescription) drugs are not required to list most inactive ingredients. Over-the-counter drugs, however, are now required to list all inactive ingredients. That requirement, effective February 1998, came about in the FDA Modernization Act of 1997 (FDAMA). For several years prior to 1998, listing inactive ingredients on the labels of over-the-counter products had been carried out on a voluntary basis through the Consumer Health Products Association's voluntary codes and guidelines.

Page 45, Last Paragraph

Comments on labeling requirements are not germane to the propylene glycol toxicity discussion, and current drug labeling requirements are noted in the preceding comment. The few cases reviewed in the Draft Report mostly included administration of massive over-doses of medication outside of labeled uses (administration to infants of megadoses of vitamin solutions labeled for children 11 years of age or above).

Pages 56 and 57

Inaccurate descriptions of data are given and improper conclusions are drawn based on the inaccurate descriptions. Data reported (and adequately described in an audited report) are not of uncertain quality: 'Due to the uncertainty of the quality of these data'.

Page 62

There are 4 well-reported studies in 4 different species, conducted on behalf of a US regulatory agency that demonstrate no developmental toxicity caused by administration of multi-gram doses of propylene glycol. Additionally there are data from a more recent, GLP guideline developmental toxicity study in mice, again demonstrating no developmental toxicity caused by multi-gram doses of propylene glycol. It is not defensible to conclude that the available data are insufficient to evaluate the developmental toxicity of propylene glycol

Page 64

Missing section on Utility of rat breeding studies. Even though these studies were conducted before GLP guidelines were developed, certainly they provide perspective on potential reproductive toxicity of propylene glycol. There were no effects identifiable, based on data provided in Table 4-1, in 3 generations of dietary administration of up to at least 10% propylene glycol. Again, this is 2-fold higher than what is currently acceptable as a maximum dose. Then offspring from the 3rd generation were administered dietary propylene glycol for another 3 generations, with no or only minimally discernable effects up to at least 10% propylene glycol. Just because data are old does not mean that they are not useful.

Page 66, Paragraph 5

Strength/Weaknesses: The statement that the findings from the rat studies were inconclusive is incorrect. The authors reported no effects up to at least 10% propylene glycol in the diet over 3

generations of administration. The Draft Report concludes that this is inconclusive, but these data, at a minimum, are supportive of the NTP mouse study, which also demonstrated no reproductive effects following several generations of daily administration of 5% propylene glycol in drinking water.

Utility: Section missing in report.

Page 67, Paragraph 2

The summary conclusion that data reported by Guerrant (reference 117) are inconclusive is not an accurate statement. At a minimum, the data support the lack of effects demonstrated in the mouse by NTP.

6. References

N.B. – In the text of the PO/PG Panel's comments, publications already included in the Draft Expert Panel Report are referred to using the reference number used in the Draft Report. The references given below are not included in the Draft Report.

Basketter, D.A. *et al*. (2000). Use of the local lymph node assay for the estimation of relative contact allergenic potency. *Contact Dermatitis* 42: 344 - 348.

Behrman, R.E., Kliegman, R., and Jenson, H.B. (2000). Nelson Textbook of Pediatrics, 16th Ed. W.B. Saunders Co., 2414 p.

Braunwald E. *et al.* (2001). Harrison's Principles of Internal Medicine. 15th ed., New York, Mc-Graw-Hill.

Chemical Business, Nov. 1992, p. 36

Chemical Marketing Reporter, Vol. 249, No. 7 p. 37, Feb. 12, 1996.

Cosmetic Ingredient Review Expert Panel (CIR) (1994). Final report on the safety assessment of propylene glcyol and polypropylene glycols. *Journal of the American College of Toxicology*. 13(6):437-491.

Descotes, J. (1988). Identification of contact allergens: the mouse ear sensitization assay. *J. Toxicol. - Cut. & Ocular Toxicol*. 7: 263 - 272.

Funk, J.O. and Maibach, H.I. (1994). Propylene glycol dermatitis: re-evaluation of an old problem. *Contact Dermatitis*; 31; 236-241.

Driscoll, C.D., Kubena, M.F., and Neeper-Bradley, T.L. (1993). *Propylene Glycol: Developmental Toxicity Gavage Study III in CD-1 Mice*. Bushy Run Research Center (BRRC), Union Carbide Chemicals and Plastics Company, Inc., 6702 Mellon Road, Export, PA, 15632-8902 (Robust Summary included as Attachment B).

Joint FAO/WHO Expert Committee on Food Additives (JECFA) (2002). *Evaluation of certain food additives and contaminants: fifty-seventh report of the Joint FAO/WHO Expert Committee on Food Additives*. WHO technical report series no. 909. Rome, Italy (available at http://www.who.int/pcs/jecfa/jecfa.htm).

Kirk-Othmer Encyclopedia of Chemical Technology, 3rd Ed., Volumes 1-26, NY, John Wiley and Sons 1978-1984

Lucas, C.D., Putnam, J.M., and Hallagan, J.B. (1999). *Flavor and Extract Manufacturers' Association of the Unites States 1995 Poundage and Technical Effects Update Survey*. Flavor and Extract Manufacturers' Association, Washington, D.C., 312 p.

Moline, J.M., Golden, A.L., Highland, J.H., Wilmarth, K.R., and Kao, A.S. (2000). *Health Effects Evaluation of Theatrical Smoke, Haze, and Pyrotechnics*. Prepared for Equity-League Pension and Health Funds by Environ International Corporation (available at http://www.equityleague.org/health/health_smokehaze.html).

National Institute for Occupational Safety and Health (NIOSH) (1983): National Occupational Exposure Survey (NOES): Final Report. Contract Number 210-80-6057, Cincinnati, Ohio.

National Institute for Occupational Safety and Health (NIOSH) (1994). *Health Hazard Evaluation Report*. HETA 90-355-2449. Actors' Equity Association/The League of American Theaters and Producers, Inc., New York, New York.

Norbäck, D., Wieslander, G., and Edling, C. (1995). Occupational exposure to volatile organic compounds (VOCs), and other air pollutants from the indoor application of water-based paints. *Ann. Occup. Hyg*. 39, 783-794.

Peltzer, M.A. and Schardein, J.L. (1966). A convenient method for processing fetuses for skeletal staining. *Stain Technology* 41:300-302.

Quast, J.F., Humiston, C.G., Wade, C.E. Beyer, J.E., Albee, R.R., Schuetz, D.J. and Morden, D.C. (1979). *Results of a toxicology study in cats fed diets containing propylene glycol for up to three months.* Unpublished report, The Dow Chemical Company, Midland, MI. (Robust Summary included as Attachment C).

Staples, R.E. (1974). Detection of visceral alterations in mammalian fetuses. *Teratology*, A37.

Townsend, C.M., Beauchamp, D.R., Evers, M.B., Mattox, K.L, and Sabiston, D.C. (2001). Sabiston Textbook of Surgery: The Biological Basis of Modern Surgical Practice, 16th Ed. W.B. Saunders Co., 1750 p.

Wilson, J.G. (1965). Embryological considerations in teratology. *In*: Teratology Principles and Techniques, J.G. Wilson and J. Warkany (eds.). The University of Chicago Press, Chicago, IL, 251-277.

U.S. NATIONAL TOXICOLOGY PROGRAM (NTP)
CENTER FOR THE EVALUATION OF RISKS TO HUMAN REPRODUCTION (CERHR)

NTP-CERHR EXPERT PANEL REPORT ON THE REPRODUCTIVE AND DEVELOPMENTAL TOXICITY OF PROPYLENE GLYCOL

DECEMBER 2002

NTP-CENTER-PG-02

ATTACHMENT A

REVIEW BY

MARK M. UDDEN, M.D.
BAYLOR COLLEGE OF MEDICINE

COMMENTS SUBMITTED: JANUARY 23, 2003
SCHEDULED EXPERT PANEL REVIEW DATE: FEBRUARY 11 - 13, 2003

Comments on NTP-CERHR Expert Panel Report on the Reproductive and Developmental Toxicity of Propylene Glycol

Mark M. Udden, MD

Associate Professor of Medicine

Hematology/Oncology Section

Department of Internal Medicine

Baylor College Of Medicine

One Baylor Plaza

Houston, Texas 77030

Telephone: 713-798-6105

E-mail: udden@bcm.tmc.edu

Executive Summary

A review of the literature on propylene glycol (PG) and its effects on red cells of humans and animals is presented. Also discussed are case reports of human exposure to high levels of PG. Hemolysis has been described when PG is administered at high concentrations as an intravenous infusion. Because PG is a highly membrane permeable solute, an osmotic lysis of red blood cells is expected. However, this hemolysis is observed only when the PG content of the infusate is 30% or more and may be further diminished if saline is substituted for water in the formulation. Hemolysis in animals receiving PG by infusion was also dependent upon injection time. Hemolysis has been described in patients receiving infusions of drugs containing high concentrations (>30%) PG as a vehicle.

A very mild reduction in hemoglobin has been observed in rats and dogs fed diets containing up to 20% PG. Cats appear to be uniquely susceptible to the effects of dietary PG. Because of their inherently unstable hemoglobin they develop a mild Heinz body hemolytic anemia. However, in rats and dogs, dietary content of as much as 8% PG is well tolerated. The relevance to humans of this sort of dietary challenge is doubtful.

The administration of PG containing drugs is generally safe. Patients have been described in the literature that developed depressed mentation, seizures, hyperosmolar states, or lactic acidosis after administration of drugs or skin treatments containing PG. Aside from those who intentionally or accidentally ingested a large amount of PG, toxicity described could be related to decreased renal clearance of PG associated with kidney disease or low birth weight of a neonate. PG joins a large number of therapeutic agents with significant renal excretion that require careful monitoring in the setting of renal insufficiency.

There is also a large body of work describing the utility of PG as a cryoprotectant for a wide variety of cell types.

The risk assessment of PG should take into consideration the following points:

(1) Effects of red blood cells have only been described in the circumstance of IV infusions in which hypertonic solutions of PG come into direct contact with red cells or in animals receiving a very large component of their dietary intake as PG.

(2) Reports of human toxicity suggest mainly a need to carefully monitor critically ill patients—in particular those with diminished renal function.

(3) Reproductive risk is low considering the successful use of PG cryopreserved sperm and oocytes.

I. Hemolysis Associated with Propylene Glycol:

A. Hemolysis in humans:

Propylene glycol (PG) is a simple solute which is highly membrane permeable. It is intermediate between glycerol and ethylene glycol in its ability to hemolyze cells when red blood cells are incubated in an isotonic solution in water[1]. Hemolysis occurs because PG is so membrane permeable that it penetrates the red blood cell bringing water rapidly into the intracellular compartment. Cell swelling ensues with resulting osmotic lysis. This effect is characteristic of many simple membrane solutes such as urea. Red blood cells incubated with PG in distilled water are rapidly hemolyzed whereas incubation with PG in normal saline produces less hemolysis. Although initially described as a completely protective effect[2], subsequent work showed that normal saline reduces hemolysis of a 30% solution but does not eliminate it totally[3]. This difference occurs because the presence of NaCl in the medium retards the flow of water into the red blood cell thereby attenuating hemolysis. Extensive studies of PG induced hemolysis of human red blood cells suggest that hemolysis occurs when the PG concentration is above 30%. Ternary solutions that also include PEG 400 produce hemolysis at lower PG concentrations, presumably because of the osmotic effect of this large molecule in the suspending media[4]. When PG is combined with ETOH hemolysis is accentuated[3]. The rate of injection of PG containing solutions also effects hemolysis[5]. From these studies it is clear that very high concentrations of PG in water infused into a vein bring red blood cells into contact with a permeable substance at high concentration thus causing lysis. Co-administration of such fluids with packed cells through the same infusion line should result in even greater hemolysis.

Hemolysis has been associated with certain drugs which require formulation with vehicles such as PG for effective intravenous administration. Etomidate, a drug used to sedate

patients in intensive care units or patients who are unprepared for intubation, is formulated with 35% PG [6]. Its administration has been associated with a number of problems including hemolysis and thrombophlebitis at the site of injection[7]. When the effect of etomidate formulated with PG was compared to formulation with HPCD a starch derived molecule a "red tinged" plasma was noted. The latter was interpreted as hemoglobin[8]. In a followup study plasma hemoglobin and haptoglobin were measured[9] in a comparison between PG and lipid formulations [8]. Volunteers receiving an infusion of etomidate containing PG did not develop anemia, but there was a significant decrease in the level of haptoglobin. Haptoglobin binds hemoglobin dimers found in plasma after intravascular hemolysis. The greater decrease in haptoglobin suggests an increase rate of intravascular hemolysis. Etomidate in PG was associated with a greater degree of free plasma hemoglobin and a greater decrement of plasma hemoglobin than etomidate in lipid emulsion. However, there was no significant effect on hematocrit or total hemoglobin other than that due to hemodilution as a result of the volume infused.

Nitroglycerin is also formulated with PG for infusion. In a group of patients in an ICU receiving nitroglycerin infusion for suspected myocardial infarction, hemolysis was said to have occurred in 3/28 patients and hemoglobinuria in one of these patients[10]. Hemolysis was indicated by elevated LDH, total bilirubin, and free plasma hemoglobin. In that study the PG content of the final infusate was 50%. In one of their patients hemolysis occurred when packed red blood cells were given through the same line as the NTG infusion.

Propylene glycol pharmacokinetics was studied in patients receiving an experimental anti-cancer drug mitoquidone (MTQ) [11]. The formulation contained polyethylene glycol 300 as well. No evidence of hemolysis was observed—in part due to use of a low dose (3 or 4.5 g/m^2)

daily of PG for 5 days and a high dose (7.5 or 15 g/m^2) on 1 day only every three weeks. The addition of a higher molecular weight polymer such as PEG 300 would also exert a protective effect because its osmolar force would retard the flow of water into the red blood cell.

It is of interest that an IV infusion of lorazepam, also formulated with PG, has been reported as the cause of hyperosmolarity and lactic acidosis in case reports without mention of concomitant hemolysis or anemia[12,13]. In some cases the absence of hemolysis could be attributed to the presence of PEG 400[14]. Reports of intoxications with PG associated with dermatologic applications, accidental ingestions, or drugs other than etomidate or NTG do not mention hemolysis or anemia as complications (see below). This suggests that IV infusion and the opportunity for direct contact of a high osmolarity solution of PG is the primary cause of hemolysis.

B. Hemolysis in animals:

Cats appear to be very sensitive to the effects of PG included as a moisturizer and source of calories in certain meals. Cats with high food intake—lactating queens and kittens are particularly susceptible. Anemia is associated with the formation of Heinz bodies—precipitates of hemoglobin resulting from either unstable hemoglobin or oxidant stress[15]. In one study cats were fed a diet containing either 12 % PG (found in commercial pet food diets, not intended for use in cats) or 41 % PG. There was a dose dependent increase in Heinz body formation with up to 92 % of erythrocytes being affected after 5 weeks of exposure to the high PG containing diet [16]. Hemolysis was further demonstrated by an increased reticulocyte count, bone marrow erythroid hyperplasia, and decreased red blood cell half-life. Another study exposing cats to PG containing diets for 16 weeks found mild changes in hematocrit, but significant decrease in red

cell life span when PG was 6 or 12 % of the diet[17]. Cat hemoglobin contains more reactive SH groups (eight) than other mammalian hemoglobins making it the hemoglobin most susceptible to oxidation-induced denaturation[18,19]. Also, the cat splenic circulation is non-sinusoidal and does not efficiently remove Heinz bodies from circulating red blood cells compared to other mammals such as humans. PG itself does not seem to induce Heinz bodies in cat erythrocytes in vitro. Potential mechanisms for this effect include either a metabolite or a change in erythrocyte metabolism induced by PG. Further work has shown that ketosis in cats is associated with Heinz body formation that may be related to metabolism of ketones. Finally, in a metabolic study, propylene glycol ingestion was shown to be the source of D-lactate accumulation in cats. The accumulation of D-lactate suggests the activity of an alternative pathway for PG metabolism that produces methylglyoxal as an intermediate[20]. Methylglyoxal, a potential oxidant, is then converted to d-lactate utilizing GSH as a co-factor. Although methylglyoxal could be the oxidant responsible for Heinz body formation, the mechanistic link between PG and Heinz body formation in the cat has not been determined.

Long term hematologic toxicity of propylene glycol in rats has also been examined[21]. In a two-year study of rats fed on up to 50,000 ppm PG (5%) showed no effect on hemoglobin or hematocrit[21]. A 13-week study of rats fed on up to 10 or 20% PG in diet did show a decrease in hemoglobin and hematocrit, but the mechanism was not elucidated[22]. A 30 day study in which rats were fed 28.4% v/v PG in standard diet showed a decrease in hemoglobin which was accompanied by a decrease in the reduced form of glutathione[23]. A similar study by the same group demonstrated changes in the lipid content of the rat red blood cell membrane following administration of PG[24]. As one of the metabolic effects of exposure to high doses of PG is alteration of the NADH/NAD ratio in cells which could change the redox state of the red cell. In

addition, a potential oxidant, methylglyoxal, is also generated during PG metabolism[25]. The generation of this oxidant might then be responsible for utilization of reduced glutathione. A single dose exposure of rats to PG produced modest changes in red blood cell surface characteristics on electron microscopy[26]. Thus it appears that the oral exposure of rats to high levels of PG can produce mild anemia, but the significance of this for human exposure is unclear. However, IV infusion of PG in rats in combination with ETOH in water demonstrated significant intravascular hemolysis[27]. Substitution of water with normal saline eliminated hemolysis for a 10% PG/30% ETOH infusate but did not affect hemolysis seen with an infusate of 20 or 30% PG formulated with 30 or 20% ETOH, respectively[27].

Dogs fed a PG containing diet for two years showed evidence of a mild decrease in hemoglobin and hematocrit accompanied by a small, but statically significant increase in reticulocyte count and bilirubin[28]. However, these effects were noted in dogs receiving 20% of their diet as PG whereas dogs on a diet that contained 8% PG had no effects whatsoever. The changes were not associated with any evidence of marrow or liver damage and were considered by the authors to be reversible. The finding of increased hemolysis with IV infusion of PG combined with ETOH and water was replicated in dogs by Fort and colleagues[27]. *In vitro* studies with dog red blood cells also showed a diminished degree of hemolysis when normal saline was substituted for water[27]. MacCannell investigated the hemodynamic response to PG in dogs and found that a 50% PG infusate in normal saline produced hemolysis which in turn had effects on mesenteric arterial flow[29]. Monkeys were exposed to air saturated with PG vapor for up to 12 months without demonstrable effects on hemoglobin or hematocrit[30]. In that study, both the control and test animals were slightly anemic due to parasitic infection at the start. Hemoglobin increased in both groups, but the increase was actually greater in the PG vapor exposed groups.

C: Importance in risk assessment:

Hemolysis is not a significant factor in the risk assessment of PG for humans for the following reasons:

1. Human effects: Hemolysis has only been observed with IV infusion of drugs with PG as a vehicle at very high concentrations of PG (35-50%), and when there is direct contact between such solutions and packed red blood cells being transfused simultaneously through a common line. Formulation with water rather than normal saline increasing such hemolysis. These effects will occur with other membrane permeable compounds such as glycerol. The addition of a large molecular weight substance such as PEG results in reduced hemolysis. Hemolysis is undoubtedly a consequence of the mixing of infusate with venous blood which brings red blood cells into direct contact with a hypertonic solution of a permeable solute. When PG is absorbed from the gut or the skin no hemolysis has been reported. It is of interest that case reports describing infusion related hemolysis are restricted to NTG or etomidate suggesting that the either the very high concentration of PG used, or the combination of PG and NTG or etomidate is responsible for hemolysis. Although there are case reports of PG effects upon administration with other compounds, these clinical experiences were not associated with hemolysis—further evidence that hemolysis is an unusual event.
2. Studies in the cat: Cats exposed to PG in the diet develop a significant Heinz body hemolysis. This cat specific effect seems to be related to the instability of feline hemoglobin. Even in the cat, the hemolysis is compensated by an increased marrow production of red blood cells, such that anemia is absent or when it exists, it is mild.

3. Studies in other species: Animal studies in rats and dogs showing an effect of PG on red blood cells require an enormous amount of PG in the diet to show an effect which in most cases is modest. In animal studies in which PG was injected intravenously, hemolysis occurred for reasons already described—the high osmolarity of the solutions formulate with or without saline. Infusion time may also be a significant issue for injection of PG. The only study of primates exposed to PG vapor suggests no effect on red blood cells.

II. Other Human Toxicity:

A. Human ingestions: There are several case reports of children[31] or adults[32,33]who developed CNS depression, lactic acidosis, or hyper osmolar states after accidental or intentional ingestion of PG. An unusual occurrence of seizures in a child with a chronic muco-cutaneous candidiasis syndrome has also been reported[34]. CNS depression was also reported in a young boy receiving massive doses of vitamin C in PG[35].

B. PG containing drug use: Drugs such as diphenylhydantoin, lorazepam, NTG, etomidate, and certain vitamins are insoluble in water and require agents such as PG for IV or oral administration[36]. Hyperosmolarity has been described in one infant[37]; in also in a series of low birth weight infants[38]. Seizures have been described in a series of low birth weight infants[39] receiving medications with high concentrations of PG. As mentioned above, lorazepam has been associated with osmolar gap metabolic acidosis[12,13]. CNS depression due to hyperosmolarity and lactic acidosis are the most common serious consequences described in these patients[33]. However, it is important to note that PG is usually well tolerated. In patients with significant renal insufficiency[10] or neonates[38] the clearance of PG is likely to be reduced. While half life of

PG in adults is 2.5-4 hours[40,41], the half life in low birth weight infants can be 19 hours[38]. In such patients the administration of PG as a vehicle must be carefully monitored by checking for osmolarity or acidosis. PG joins a whole host of therapeutic agents which also must be carefully monitored in neonates and patients with renal disease. It is significant that no deaths have been related to PG[25].

C. Dermatologic use:

Increased, but non-toxic levels of PG have been found in burn patients treated with silver sulfadiazine formulated with PG[42]. Burn patients treated with up to 6.1 g/kg/24 hrs had no significant increase in osmolarity or lactic acid production[43]. A toxic level was found in an eight month old infant with toxic epidermal necrolysis involving 78% of his body surface area[44]. Skin absorption of PG is usually minimal when the dermis is intact. Destruction of this barrier by a burn allows for free absorption of PG and hence the occurrence of hyperosmolarity and lactic acidosis in such patients. PG does not appear to be a significant dermatologic toxin, although minor sensitization to PG has been reported[45,46].

D. Relevance to human exposure:

PG appears to be a safe and relatively innocuous substance that has many useful applications in drug formulations. However, any drug has a therapeutic index representing the difference between a dose that produces a desired effect compared to a toxic effect. Some drugs, such as digoxin, have a relatively narrow therapeutic index and clinicians are aware that its administration requires great care. However PG administration appears to be safe over a wide range of exposures. It can produce toxicity by virtue of its osmolar effects and its metabolism to lactate. To date the reported incidents of this toxicity are restricted to very special circumstances: neonates, burn victims, and patients in intensive care who usually have

concomitant renal compromise. In neonates and renal failure patients a prolonged excretion of PG is expected. A recent review of the toxicity of drug vehicles for pediatric patients emphasizes the need for adequate labeling of these compounds which include sulfites, benzalkonium chloride, benzyl alcohol, as well as PG[47]. Also it should be remembered that this type of exposure occurs under the watchful eyes of ICU teams and other specialized caregivers and that treatment with hemodialysis or simple withdrawal of the IV agent has been successful in management of these patients.

III. Reproductive Toxicity:

A. Use of PG as a cryopreservative:

A Medline search for propylene glycol related papers produced over one hundred and fifty articles describing the use of PG in cryopreservation of a wide range of tissues and fifty of these describe use for human tissues. These include platelets[48,49], sperm[50,51], oocytes[52], embryos[53], and stem cells[54]. The rapid permeability of PG through membranes is an advantage in protecting cells from damage due to formation of ice crystals during cryopreservation. The viability and subsequent successful use of these cells, in particular for sperm and oocytes, further supports the low toxicity of PG as a reproductive toxin.

Reference List

(1) Jay AW, Rowlands S. The stages of osmotic haemolysis. J Physiol. 1975;252:817-832.

(2) Weatherby JH, Haag HB. Toxicity of propylene glycol. J Am Pharmaceutical Assoc. 1938;27:466-471.

(3) Reed KW, Yalkowsky SH. Lysis of human red blood cells in the presence of varous cosolvents. J Parenter Sci Technol. 1984;39:64-69.

(4) Ku SH, Cadwallader DE. Behavior of erythrocytes in ternary solvent systems. J Pharm Sci. 1975;64:1818-1821.

(5) Obeng EK, Cadwallader DE. In vitro dynamic method for evaluating the hemolytic potential of intravenous solutions. J Parenter Sci Technol. 1989;43:167-173.

(6) Bergen JM, Smith DC. A review of etomidate for rapid sequence intubation in the emergency department. J Emerg Med. 1996;15:221-230.

(7) Doenicke A, Roizen MF, Nebauer AE et al. A comparison of two formulations for etomidate, 2-hydroxypropyl-beta-cyclodextrin (HPCD) and propylene glycol. Anesth Analg. 1994;79:933-939.

(8) Doenicke A, Roizen MF, Hoernecke R et al. Haemolysis after etomidate: comparison of propylene glycol and lipid formulations. Br J Anaesth. 1997;79:386-388.

(9) Nebauer AE, Doenicke A, Hoernecke R, Angster R, Mayer M. Does etomidate cause haemolysis? Br J Anaesth. 1992;69:58-60.

(10) Demey HE, Daelemans RA, Verpooten GA et al. Propylene glycol-induced side effects during intravenous nitroglycerin therapy. Intensive Care Med. 1988;14:221-226.

(11) Speth PA, Vree TB, Neilen NF et al. Propylene glycol pharmacokinetics and effects after intravenous infusion in humans. Ther Drug Monit. 1987;9:255-258.

(12) Arbour R, Esparis B. Osmolar gap metabolic acidosis in a 60-year-old man treated for hypoxemic respiratory failure. Chest. 2000;118:545-546.

(13) Arbour RB. Propylene glycol toxicity related to high-dose lorazepam infusion: case report and discussion. Am J Crit Care. 1999;8:499-506.

(14) Tayar J, Jabbour G, Saggi SJ. Severe hyperosmolar metabolic acidosis due to a large dose of intravenous lorazepam. N Engl J Med. 2002;346:1253-1254.

(15) Christopher MM, Perman V, Eaton JW. Contribution of propylene glycol-induced Heinz body formation to anemia in cats. J Am Vet Med Assoc. 1989;194:1045-1056.

(16) Christopher MM, White JG, Eaton JW. Erythrocyte pathology and mechanisms of Heinz body-mediated hemolysis in cats. Vet Pathol. 1990;27:299-310.

(17) Bauer MC, Weiss DJ, Perman V. Hematological alterations in kittens induced by 6 and 12% dietary propylene glycol. Vet Hum Toxicol. 1992;34:127-131.

(18) Hamilton MN, Edelstein SJ. Cat hemoglobin. pH dependence of cooperativity and ligand binding. J Biol Chem. 1974;249:1323-1329.

(19) Hamilton MN, Edelstein SJ. Cat hemoglobin: pH-dependent cooperativity of oxygen binding. Science. 1972;178:1104-1106.

(20) Christopher MM, Eckfeldt JH, Eaton JW. Propylene glycol ingestion causes D-lactic acidosis. Lab Invest. 1990;62:114-118.

(21) Gaunt IF, Carpanini FM, Grasso P, Lansdown AB. Long-term toxicity of propylene glycol in rats. Food Cosmet Toxicol. 1972;10:151-162.

(22) Okumura M, Yamada S, Ito N, Kawamura N, Hiramatsu R. Subacute toxicity of propylene glycol in F-344 rats. Aichi Eishoho. 1984;34:27-34.

(23) Amma MKP, Namagiri T, Chum A. The redox state of propane-1,2-diol fed rat erythrocytes. Res Bull Panjab Univer. 1984;35:109-113.

(24) Ahluwalia P, Amma MKP. Hypolipidemic effect of propane-1,2-diol on the morphology of rat erythrocytes. Res Bull Panjab Univer. 1984;35:157-159.

(25) LaKind JS, McKenna EA, Hubner RP, Tardiff RG. A review of the comparative mammalian toxicity of ethylene glycol and propylene glycol. Crit Rev Toxicol. 1999;29:331-365.

(26) Sainl M, Amma MKP, Dash S, Nagpaul JP. Hematological alterations in propylene glyco-dosed female rats are minimal. Vet Hum Toxicol. 1996;38:81-85.

(27) Fort FL, Heyman IA, Kesterson JW. Hemolysis study of aqueous polyethylene glycol 400, propylene glycol and ethanol combinations in vivo and in vitro. J Parenter Sci Technol. 1984;38:82-87.

(28) Weil CS, Woodside MD, Smyth HF, Jr., Carpenter CP. Results of feeding propylene glycol in the diet to dogs for two years. Food Cosmet Toxicol. 1971;9:479-490.

(29) MacCannell K. Hemodynamic responses to glycols and to hemolysis. Can J Physiol Pharmacol. 1968;47:563-569.

(30) Robertson OH, Loosli CG, Puck TT et al. Tests for the chronic toxicity of propylene glycol and triethylene glycol on monkeys and rats by vapor inhalation and oral administration. J Pharmacol Exper Therap. 1947;91:52-76.

(31) Glover ML, Reed MD. Propylene glycol: the safe diluent that continues to cause harm. Pharmacotherapy. 1996;16:690-693.

(32) Cate JC, Hedrick R. Propylene glycol intoxication and lactic acidosis. N Engl J Med. 1980;303:1237.

(33) Lolin Y, Francis DA, Flanagan RJ, Little P, Lascelles PT. Cerebral depression due to propylene glycol in a patient with chronic epilepsy--the value of the plasma osmolal gap in diagnosis. Postgrad Med J. 1988;64:610-613.

(34) Arulanantham K, Genel M. Central nervous system toxicity associated with ingestion of propylene glycol. J Pediatr. 1978;93:515-516.

(35) Martin G, Finberg L. Propylene glycol: a potentially toxic vehicle in liquid dosage form. J Pediatr. 1970;77:877-878.

(36) Kelner MJ, Bailey DN. Propylene glycol as a cause of lactic acidosis. J Anal Toxicol. 1985;9:40-42.

(37) Huggon I, James I, Macrae D. Hyperosmolality related to propylene glycol in an infant treated with enoximone infusion. BMJ. 1990;301:19-20.

(38) Glasgow AM, Boeckx RL, Miller MK et al. Hyperosmolality in small infants due to propylene glycol. Pediatrics. 1983;72:353-355.

(39) MacDonald MG, Getson PR, Glasgow AM et al. Propylene glycol: increased incidence of seizures in low birth weight infants. Pediatrics. 1987;79:622-625.

(40) Kolloffel WJ, Weekers LE, Goldhoorn PB. Pharmacokinetics of propylene glycol after rectal administration. Pharm World Sci. 1996;18:109-113.

(41) Yu DK, Elmquist WF, Sawchuk RJ. Pharmacokinetics of propylene glycol in humans during multiple dosing regimens. J Pharm Sci. 1985;74:876-879.

(42) Kulick MI, Wong R, Okarma TB, Falces E, Berkowitz RL. Prospective study of side effects associated with the use of silver sulfadiazine in severely burned patients. Ann Plast Surg. 1985;14:407-419.

(43) Commens CA. Topical propylene glycol and hyperosmolality. Br J Dermatol. 1990;122:77-80.

(44) Fligner CL, Jack R, Twiggs GA, Raisys VA. Hyperosmolality induced by propylene glycol. A complication of silver sulfadiazine therapy. JAMA. 1985;253:1606-1609.

(45) Catanzaro JM, Smith JG, Jr. Propylene glycol dermatitis. J Am Acad Dermatol. 1991;24:90-95.

(46) Hannuksela M, Forstrom L. Reactions to peroral propylene glycol. Contact Dermatitis. 1978;4:41-45.

(47) "Inactive" ingredients in pharmaceutical products: update (subject review). American Academy of Pediatrics Committee on Drugs. Pediatrics. 1997;99:268-278.

(48) Arnaud FG, Pegg DE. Cryopreservation of human platelets with propane-1,2-diol. Cryobiology. 1990;27:130-136.

(49) Arnaud FG, Hunt CJ, Pegg DE. Some effects of propane-1,2-diol on human platelets. Cryobiology. 1990;27:119-129.

(50) Gilmore JA, Liu J, Woods EJ, Peter AT, Critser JK. Cryoprotective agent and temperature effects on human sperm membrane permeabilities: convergence of theoretical and empirical approaches for optimal cryopreservation methods. Hum Reprod. 2000;15:335-343.

(51) Gilmore JA, Liu J, Gao DY, Critser JK. Determination of optimal cryoprotectants and procedures for their addition and removal from human spermatozoa. Hum Reprod. 1997;12:112-118.

(52) Wininger JD, Kort HI. Cryopreservation of immature and mature human oocytes. Semin Reprod Med. 2002;20:45-49.

(53) Ben Ozer S, Vermesh M. Full term delivery following cryopreservation of human embryos for 7. 5 years. Hum Reprod. 1999;14:1650-1652.

(54) Woods EJ, Liu J, Derrow CW et al. Osmometric and permeability characteristics of human placental/umbilical cord blood CD34+ cells and their application to cryopreservation. J Hematother Stem Cell Res. 2000;9:161-173.

U.S. NATIONAL TOXICOLOGY PROGRAM (NTP)
CENTER FOR THE EVALUATION OF RISKS TO HUMAN REPRODUCTION (CERHR)

NTP-CERHR EXPERT PANEL REPORT ON THE REPRODUCTIVE AND DEVELOPMENTAL TOXICITY OF PROPYLENE GLYCOL

DECEMBER 2002

NTP-CENTER-PG-02

ATTACHMENT B

ROBUST SUMMARY OF

Driscoll, C.D., Kubena, M.F., and Neeper-Bradley, T.L. (1993).
Propylene Glycol: Developmental Toxicity Gavage Study III in CD-1 Mice
Bushy Run Research Center (BRRC)
Union Carbide Chemicals and Plastics Company, Inc.
6702 Mellon Road, Export, PA, 15632-8902

COMMENTS SUBMITTED: JANUARY 23, 2003
SCHEDULED EXPERT PANEL REVIEW DATE: FEBRUARY 11 - 13, 2003

I U C L I D

Data Set

Existing Chemical	:	ID: 57-55-6
CAS No.	:	57-55-6
EINECS Name	:	propane-1,2-diol
EC No.	:	200-338-0
TSCA Name	:	1,2-Propanediol
Molecular Formula	:	C3H8O2

Producer related part

Company	:	Propylene Oxide/Propylene Glycol Panel
Creation date	:	18.01.2003

Substance related part

Company	:	Propylene Oxide/Propylene Glycol Panel
Creation date	:	18.01.2003
Status	:	
Memo	:	
Printing date	:	21.01.2003
Revision date	:	
Date of last update	:	20.01.2003
Number of pages	:	4
Chapter (profile)	:	Chapter: 5.8.2
Reliability (profile)	:	Reliability: without reliability, 1, 2, 3, 4
Flags (profile)	:	Flags: without flag, confidential, non confidential, WGK (DE), TA-Luft (DE), Material Safety Dataset, Risk Assessment, Directive 67/548/EEC, SIDS

5.8.2 DEVELOPMENTAL TOXICITY/TERATOGENICITY

Species	:	mouse
Sex	:	female
Strain	:	CD-1
Route of admin.	:	gavage
Exposure period	:	GD 6-15
Frequency of treatm.	:	Once per day
Duration of test	:	Sacrifice on GD18
Doses	:	0 (water), 0.5, 5.0 or 10.0 ml/kg bwt/d (equivalent to 0, 518, 5180 or 10360 mg/kg bwt/d)
Control group	:	yes, concurrent vehicle
NOAEL maternal tox.	:	> 10 ml/kg bw
NOAEL teratogen.	:	> 10 ml/kg bw
NOAEL Fetotoxicity	:	> 10 ml/kg bw
Result	:	No abnormalities found
Method	:	Similar to OECD 414 Guideline
Year	:	1993
GLP	:	yes
Test substance	:	Propylene glycol

Method :

Animals and treatment
Propylene glycol (99.9% purity) was administered by gavage to CD-1® mice (30/group; age 60-65 d at mating) from gestation day (GD) 6 thru 15 (GD 0 = day of sperm positive finding). The test article was administered undiluted and the dose levels were 0.5, 5.0, or 10 ml/kg/day (518, 5,180, or 10,360 mg/kg/day) with the vehicle control receiving water at the same dose volume as the high-dose group.

Maternal observations
Clinical observations were collected daily (twice during the dosing period) and maternal body weights, feed, and water consumption was measured at three-day intervals (that is, GD0, 6, 9, 12, 15, 18).

Maternal observations at necropsy
The dams were euthanized and weighed on GD 18. The uterus, ovaries (including corpora lutea: CL), cervix, vagina and organs within the peritoneal and thoracic cavities were examined grossly. The liver, kidney and gravid uterus were weighed. The number of corpora lutea, and the number and status of implantation sites, was determined.

Fetal observations
All live and dead fetuses were weighed, sexed, and examined externally for malformations (including cleft palate) and variations. Live and dead fetuses and early and late resorptions were counted and recorded. All live fetuses were examined for thoracic and abdominal malformations and variations by the method of Staples (1974). One-half of the liver fetuses were decapitated, heads fixed in Bouin's solution, and craniofacial structures examined by the method of Wilson (1965). All fetuses were eviscerated, fixed in ethanol, and processed for skeletal examination. Alizarin red S was used to stain the skeleton for examination (Peltzer and

Schardein, 1966).

Statistical analysis
The statistical unit for comparison was the litter. The data for quantitative continuous variables were intercompared for the 3 treatment groups and the controls using Levene's test for equality of variance, ANOVA and T-tests. Non-parametric data were evaluated using the Kruskal-Wallis test, followed by the Mann-Whitney U test when appropriate. Incidence data were compared using Fisher's Exact Test.

Remark :

Additional references:
Peltzer, MA and Schardein, JL (1966) A convenient method for processing fetuses for skeletal staining. Stain Technology, 41: 300-302.

Staples, RE (1974) Detection of visceral alterations in mammalian fetuses. Teratology, A37.

Wilson, JG (1965) Embryological considerations in teratology. In: Teratology Principles and Techniques, JG Wilson and J Warkany, eds. The University of Chicago Press, Chicago, IL, pp251-277.

Result :

Maternal observations
No females aborted or died or were removed from the study. There were no treatment related clinical signs, nor were any effects reported on body weights, weight gain, rate of weight gain, or feed consumption. Water consumption was increased significantly in both the 5 ml/kg/day (approx. 20%) and 10 ml/kg/day groups (approx. 35%) on GD 12-15. There were no treatment-related findings in any of the parameters collected at necropsy.

One female from the 0.5 and one from the 10 ml/kg/day groups had only early resorptions at necropsy. Nonpregnant females in the control, low, mid, and high-dose groups numbered 2, 1, 2, and 0, respectively; the pregnancy rate ranged from 93.3 to 100%. 28-29 litters/group were available for examination.

Fetal observations
There were 28 to 29 live litters per group available for examination at necropsy.

There was no statistically significant differences in male or female fetal body weights between the treated and control groups. However, when the fetal body weight data for the sexes were combined, there was a 3% decrement (statistically significant) in fetal body weights in the 10 ml/kg/day group when compared to the control group values. The authors considered this decrement in body weight not to be biologically significant due to the small magnitude of the change and the fact that the low dose group had similar fetal body weights (lack of an increase in response with a 20-fold increase in dose level between the low and high dose groups).

There were no treatment related differences in the total number of implantations, number of viable or nonviable implants, or sex ratios. The incidence of external, visceral, and skeletal malformations/variations was comparable between the treated and control groups.

An increase in a variation (unossified cervical centra #1, #2, #3, #4) in the 10 ml/kg/day group was not considered biologically significant since the incidence was within the historical control litter incidence range (7 - 23%).

Two unrelated findings in the low-dose group were not considered treatment related due to the lack of dose response relationship.

The authors note that they expected 4 statistically significant changes in the 86 individual endpoints evaluated.

Conclusion **:**

The authors concluded that 10 ml/kg/day (10,360 mg/kg/day) was the No Observed Effect Level (NOEL) for developmental toxicity.

Reliability **:** (1) valid without restriction
20.01.2003 (1)

(1) Driscoll, CD, Kubena, MF and Neeper-Bradley, TL (1993) Propylene glycol: developmental toxicity study III in CD-1 mice. Bushy Run Research Center (BBRC), Union Carbide Chemicals and Plastics Company, Inc (UCC&P), 6702 Mellon Road, Export, PA, 15632-8902.

U.S. NATIONAL TOXICOLOGY PROGRAM (NTP)
CENTER FOR THE EVALUATION OF RISKS TO HUMAN REPRODUCTION (CERHR)

NTP-CERHR EXPERT PANEL REPORT ON THE REPRODUCTIVE AND DEVELOPMENTAL TOXICITY OF PROPYLENE GLYCOL

DECEMBER 2002
NTP-CENTER-PG-02

ATTACHMENT C

ROBUST SUMMARY OF

Quast, JF, Humiston, CG, Wade, CE, Beyer, JE, Albee, RR, Schuetz, DJ and Morden, DC (1979)
Results of a toxicology study in cats fed diets containing propylene glycol for up to three months.
Unpublished report, The Dow Chemical Company, Midland, MI.

COMMENTS SUBMITTED: JANUARY 23, 2003
SCHEDULED EXPERT PANEL REVIEW DATE: FEBRUARY 11 - 13, 2003

I U C L I D

Data Set

Existing Chemical	:	ID: 57-55-6
CAS No.	:	57-55-6
EINECS Name	:	propane-1,2-diol
EC No.	:	200-338-0
TSCA Name	:	1,2-Propanediol
Molecular Formula	:	C3H8O2

Producer related part

Company	:	Propylene Oxide/Propylene Glycol Panel
Creation date	:	21.01.2003

Substance related part

Company	:	Propylene Oxide/Propylene Glycol Panel
Creation date	:	21.01.2003
Status	:	
Memo	:	Chapter 5.4: Quast et al., 1979
Printing date	:	21.01.2003
Revision date	:	
Date of last update	:	21.01.2003
Number of pages	:	65
Chapter (profile)	:	Chapter: 5.4
Reliability (profile)	:	Reliability: without reliability, 1, 2, 3, 4
Flags (profile)	:	Flags: without flag, confidential, non confidential, WGK (DE), TA-Luft (DE), Material Safety Dataset, Risk Assessment, Directive 67/548/EEC, SIDS

5.4 REPEATED DOSE TOXICITY

Species	:	cat
Sex	:	male
Route of admin.	:	oral feed
Exposure period	:	69 - 94 days
Frequency of treatm.	:	daily
Doses	:	80, 443, 675, 1763, 4239 mg/kg bw/d
Control group	:	yes, concurrent vehicle
NOAEL	:	= 80 mg/kg bw
LOAEL	:	= 443 mg/kg bw
Method	:	other: investigative study
Year	:	1979
GLP	:	no
Test substance	:	Propylene glycol

Method :

Animals and treatments
This investigation proceeded in two phases. In phase 1 groups of two male cats were fed commercial diet with added propylene glycol designed to deliver 500 or 5000 mg/kg bw/day. In phase 2, groups of two males were fed diets designed to deliver 100 or 1000 mg/kg bw/day. The phase 2 investigation also included 2 cats fed a standard domestic cat diet containing approx 7% propylene glycol as humectant. Water was available ad libitum.

Diet preparation
A pre-determined amount of test substance was mixed directly with 125 g of diet, this being an amount which the animals were expected to consume within 1 hr. The amount of propylene glycol actually consumed by the animals was calculated based on the amount of diet eaten. Additional (unsupplemented) food was made available as necessary.

Observations
The cats were observed daily for demeanour and clinical signs. Body weights were recorded twice weekly, and used to calculate the amount of propylene glycol to be added to the test diets.

Clinical chemistry
Serum urea nitrogen concentration, alkaline phosphatase, glutamic pyruvic transaminase, glutamic oxaloacetic transaminase, total bilirubin and glucose were determined on blood collected from the jugular vein on 3 occasions before treatment commenced and on 4 - 10 occasions during the study.

Hematology
Packed cell volume, red cell counts, total and differential white blood cell count, hemoglobin concentration, methemoglobin concentration, reticulocyte count, Heinz body count and osmotic fragility were determined on 2 or 3 occasions before treatment commenced and on 5 - 11 occasions during the study.

Urinanalysis
A sample of urine was aspirated from the bladder at necropsy, and examined for specific gravity, pH, glucose, protein, ketones, bilirubin, occult blood and urobilinogen.

Necropsy and histopathological procedures
Animals were fasted overnight and sacrificed by exsanguination following barbiturate anesthesia. A gross pathological examination was performed on the eyes and internal organs. Liver, kidney, heart, brain and testes weights were determined. A comprehensive range of organs, along with any tissue which appeared abnormal, were sampled and processed (H&E staining) for subsequent microscopic evaluation. In addition liver, spleen and vertebral bone with bone marrow were stained with Prussian blue stain (to demonstrate iron), while liver sections were also stained with Periodic Acid Schiff reagent (glycogen). A total of 10 tissues were subject to histopathological examination.
Electron microscopy
Fixed peripheral erythrocytes and sections of liver were stained with toluidine blue (thick sections) or uranyl acetate and lead citrate (thin sections) from one animal from the control and each of the treatment groups.

Statistics
Differences between the groups in final body weight and absolute and relative organ weight values were evaluated using one-way analysis of variance, with differences between the treated and control groups examined using Dunnett's test. No statistical analysis was performed on the other data due to the small group sizes.

Remark :

Heinz body formation was the key finding in this investigation. This occurred with no evidence of accompanying hemolytic anemia such as that commonly reported in cats and other species following treatment with aromatic amino or nitro compounds. This indicates a differential mechanism of action for propylene glycol. With the exception of increased hemosiderin deposits in liver and spleen (secondary to Heinz body formation), no other treatment-related systemic toxicity was seen in cats at treatment levels up to 4239 mg/kg bw/d for 94 days.

Result :

General
Results from the two investigations have been combined in this summary for ease of presentation and interpretation.

The amount of food consumed by the cats was slightly lower than anticipated, leading to average achieved treatment levels of 80, 443, 675 and 4239 mg/kg bw/day. Animals fed the domestic diet received a calculated average daily dose of 1763 mg/kg bw/day.

There were no adverse changes in demeanour or clinical signs, body weight gain or clinical chemistry parameters in any of the treatment groups.

Pathology
No abnormalities of the eyes or internal organs were reported. Organ

weight determinations showed a large degree of variability, presumably reflecting the small group sizes used. Spleen and testis weights were particularly affected but since there was no apparent dose-related trend, and since similar variability was present in the controls, these findings were considered unrelated to propylene glycol treatment.

Histopathology
Treatment-related changes consisted of a slight increase in the amount of hemosiderin in individual Kupffer cells of the liver and in reticuloendothelial cells from the higher treatment groups, particularly those animals given 5000 mg/kg/d. With the exception of hemosiderin deposition, no other treatment-related abnormalities were present in liver from any animals given propylene glycol.

Examination of testis tissue revealed increased numbers of multinucleated giant cells in seminiferous tubules from one of the two animals given 100, 500 and 5000 mg/kg/day or the domestic diet, but not in animals given 1000 mg/kg/d. In one animal from the 5000 mg/kg/d group the testes were hypoplastic. These findings were considered by the investigators to reflect differences in sexual development between the animals rather than any treatment-specific effect.

There were no treatment-related microscopic observations in any of the other tissues examined in this study.

Electron microscopy
The ultrastructural appearance of Heinz bodies from cats given propylene glycol were essentially as expected, and did not suggest any unusual etiology. Ultrastructural changes in Kupffer cells were consistent with increased hemosiderin deposition. Electron microscopy confirmed an absence of any other changes in the liver of treated animals.

Hematological parameters
General hematology
PCV, RBC, HgB, WBC and differential WBC data showed a large degree of variation during the pretest- and test periods in both the control and treated animals. Against this background, there was no evidence of any propylene glycol-related adverse effects.

Heinz bodies
In contrast to other blood parameters, Heinz body determinations demonstrated a clear response to propylene glycol treatment. The incidence of Heinz bodies increased in cats from the 5000 mg/kg/d after 4 days treatment, and remained elevated until the end of the study. The average incidence in this group was 32% versus a pre-test and control incidence that was generally below 1%. Mean Heinz body incidence in cats fed the commercially-prepared diet (equivalent to ingestion of 1763 mg propylene glycol / kg bw / day) was 13 - 20%.

In cats from the 1000 and 500 mg/kg/d groups the Heinz bodies were smaller in size than observed in the 5000 mg/kg/d group, and were present at an average incidence of 2.5 - 6.4% and 1.5 - 3.5%, respectively. Heinz body appearance, size and incidence (0.4 - 0.7%) in the 100 mg/kg/d group

were essentially indistinguishable from the controls or the pre-test values.

Reticulocytes
Detailed examination, and re-examination, of reticulocyte count data for cats ingesting propylene glycol did not reveal a consistent treatment-related increase in the incidence of either punctate or aggregate forms. (Note : an increase in these forms (indicative of an erythrocytic regenerative response) was anticipated by the investigators.)

Methemoglobin
Comparison of control, pre-test and test results indicated there was no induction of methemoglobinaemia in cats consuming propylene glycol.

Osmotic fragility
Red cell osmotic fragility data showed a large degree of variation during the pretest- and test periods in both the control and treated animals. Against this background, there was no evidence of any propylene glycol-related adverse effect.

Conclusion : Under the conditions of this investigation in cats, the NOAEL for Heinz body formation and associated hemosiderin deposition in liver and spleen was 80 mg/kg bw/d. The NOAEL for other systemic effects was > 4239 mg/kg bw/d.

Reliability : (1) valid without restriction

21.01.2003 (1)

(1) Quast, JF, Humiston, CG, Wade, CE, Beyer, JE, Albee, RR, Schuetz, DJ and Morden, DC (1979) Results of a toxicology study in cats fed diets containing propylene glycol for up to three months. Unpublished report, The Dow Chemical Company, Midland, MI.